Characterization of mycosphaerella blight resistance, lodging resistance, and micronutrient concentration in a field pea recombinant inbred line population

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Canada

By

Yong Liu

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ABSTRACT

Field pea (*Pisum sativum* L.) production in western Canada is often negatively affected by mycosphaerella blight and lodging. Micronutrient (selenium, zinc and iron) concentration is one of the important quality traits determining the market value of field pea. Therefore, improving mycosphaerella blight resistance, lodging resistance, and micronutrient concentration are important pea breeding objectives. Moderate genetic variation for these traits have been observed in field pea germplasm. In order to map the quantitative trait loci (QTLs) associated with these traits, a population of 142 $F_{7:9}$ and $F_{7:10}$ recombinant inbred lines (RILs) derived from a cross between Carrera (susceptible to mycosphaerella blight and lodging) and CDC Striker (moderately resistant to mycosphaerella blight and lodging) were evaluated at Saskatoon and Rosthern, SK, Canada in 2010 and 2011. Over 2010 and 2011, mean area under the disease progress curve (AUDPC) of mycosphaerella blight ranged from 131 to 205 and 144 to 235 for Saskatoon and Rosthern, respectively. At physiological maturity, mean lodging ratings of the RILs ranged from 3.8 to 8.3 at Saskatoon and 4.5 to 8.5 at Rosthern. Mean selenium concentration ranged from 1.16 to 4.35 ppm at Saskatoon and 0.18 to 0.81 ppm at Rosthern. Mean zinc concentration ranged from 25.45 to 37.71 ppm at Saskatoon and from 25.08 to 38.15 ppm at Rosthern. Mean iron concentration varied from 41.85 to 58.80 ppm at Saskatoon and from 39.13 to 58.80 ppm at Rosthern. A genetic linkage map consisting of 56 simple sequence repeat (SSR) markers was generated. The total coverage of the map was 288.3 cM and the average distance between markers was 5.1 cM. A region between markers AA491 and AA278 on linkage group III was identified as harboring QTLs associated with mycosphaerella blight, lodging, zinc and iron concentration. All QTLs were derived from CDC Striker, except one associated with higher Zn concentration which was derived from Carrera. The total

phenotypic variation of each trait explained by the QTL was 18.5%, 10.1%, 11.4% and 13.1%, respectively.

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LIST OF ABBREVIATIONS

ANOVA: analysis of variance

AFLP: amplified fragment length polymorphism

AUDPC: area under the disease progress curve of mycosphaerella blight

AUDPC2010ROS: area under the disease progress curve of mycosphaerella blight at Rosthern in 2010

AUDPC2011SAS: area under the disease progress curve of mycosphaerella blight at Saskatoon in 2011

AULPC: area under the lodging progress curve

CC: stipules and stems at the seedling stage under controlled conditions

CIM: composite interval mapping

cM: centiMorgans

CS: stems at the seedling stage under controlled conditions

CTAB: hexadecyltrimethylammonium bromide

CV: coefficient of variation

DF: days to flowering

DM: days to maturity

DNA: deoxyribonucleic acid

DRl: disease rating on leaves scored under field conditions

DRseedl: disease rating in leaves of seedlings scored under growth chamber conditions

DRst: disease rating on stems scored under field conditions

DS: disease severity (percentage of the plant area covered by symptoms) estimated under field conditions

FC: stipules and stems at the adult plant stage in the field

Fe: iron

FS: stems at the adult plant stage in the field

GLM: general linear model

HT: plant height

H^2: broad-sense heritability

IM: Interval Mapping

LG: linkage group

LG1: the first rating of lodging

LG2: the second rating of lodging

LG3: the third rating of lodging

LG4: the fourth rating of lodging

LG5: the fifth rating of lodging

LOD: logarithm of odds ratio

LP: light penetration

LSD: least significant difference

MB1: the first rating of mycosphaerella blight

MB2: the second rating of mycosphaerella blight

MB32010SAS: the third rating of mycosphaerella blight scored at Saskatoon in 2010

MB32011SAS: the third rating of mycosphaerella blight scored at Saskatoon in 2011

MB32010ROS: the third rating of mycosphaerella blight scored at Rosthern in 2010

MB32011ROS: the third rating of mycosphaerella blight scored at Rosthern in 2011

MB3: the third rating of mycosphaerella blight

MB4: the fourth rating of mycosphaerella blight

MIM: multiple interval mapping

NBS: nucleotide binding site

NS: not significant

PAGE: polyacrylamide gel electrophoresis

PCR: polymerase chain reaction

PS: plant stand

QTL: quantitative trait loci

RAPD: random amplification of polymorphic DNA

RFLP: restriction fragment length polymorphism

RGA: resistance gene analogs

RILs: recombinant inbred lines

Se: selenium

SD: seed dimpling

SS: seed shape

SMA: Single marker analysis

SNP: single nucleotide polymorphism

SSR: simple sequence repeats

STS: sequence tagged sites

TSW: thousand seed weight

Yld: yield

Zn: zinc

σ^2e: error variance

σ^2g: genotypic variance

σ^2_{gl}: genotype X location interaction variance

σ^2_{gy}: genotype X year interaction variance

σ^2_{gyl}: genotype X year X location interaction variance

σ^2_{p}: phenotypic variance

1.0 INTRODUCTION

Field pea (*Pisum sativum* L.) was among the first crops cultivated and domesticated about 9,000 BC in the Fertile Crescent near the Tigris and Euphrates rivers. It is now grown in all climatic zones, including the high elevations in the tropics. It is an important protein-rich cool-season legume crop (Zohary and Hopf, 1973). On average, dry pea and vegetable pea were grown on over 6.5 and 1.1 million hectares respectively from 2003 to 2007 in the world. As well, the mean productions from 2003 to 2007 of dry pea and vegetable pea were 10.6 and 8.1 million tonnes worldwide (FAOSTAT, 2008).

Field pea grows well under fertile, well-drained soils with high moisture holding capacity (Schata and Endres, 2003) and pea adapts well to cool, semi-arid climates. Field pea seeds will germinate at a soil temperature of 4.4 ℃, and typically takes 7-10 days to emerge. Seedlings can tolerate spring frost and if injured by frost, a new shoot will emerge below the soil surface. Flowering usually takes 40 to 50 days after planting. The flowering period is normally two to four weeks, depending on the weather conditions during flowering. Pea plants are self-pollinated and each flower can produce a pod containing four to nine seeds (Schata and Endres, 2003).

Field pea is well adapted to zero-tillage systems. Pea production is often most successful when grown in rotation with cereals such as barley or wheat (McVicar et al. 2009). Cereals grown as previous crops result in low soil nitrogen and disease levels for pulses, maximizing nitrogen fixation and minimizing diseases for the growing pea crop. Virus transmission by aphids can be inhibited by standing cereal stubble (Jenkins et al. 2005).

In the early 1900s, Ontario led pea production in Canada. Since the mid 1980's, Saskatchewan has been the major province leading Canadian pea production

(Saskatchewan Pulse Grower, 2002). About 75% of Canada's peas are produced by Saskatchewan, with the remainder grown primarily in Alberta and limited production in Manitoba (Fleury, 2009).

Field pea production in western Canada is negatively affected by several fungal diseases and lodging. Variation in pea yield is frequently associated with diseases. The most important fungal disease in pea worldwide is the ascochyta blight complex, with the majority of the damage typically caused by mycosphaerella blight. All above ground portions of the pea plant can be infected by this disease, leading to numerous lesions and extended necrosis (Kraft et al. 1998). Seed per stem and seed size is reduced by mycosphaerella blight. The disease begins in the lower portion of pea plants and moves upward if weather conditions are cool and wet (Tivoli et al. 1996). Complete resistance to mycosphaerella blight is lacking in field pea germplasm, so it is a major goal to breed cultivars with improved resistance to this pathogen (Kraft et al. 1998; Banniza et al. 2005).

Lodging occurs when pea plants are unable to support the weight of the canopy and filling pods and keep their orientation in an upright position (Holland, 1990; Stelling, 1994). Pea cultivars with weak stems show severe lodging after flowering, causing reductions in forage and seed yield (Stelling, 1994). Thus, breeding for lodging resistance is important in order to increase yield and facilitate harvest procedures.

Seed quality plays an especially important role in the food pea market for nutritional purposes (McPhee, 2007). Micronutrient accumulation and uptake traits are inherited and can be improved by breeding approaches (Graham et al. 1999). Field pea cultivars grown in Saskatchewan displayed a moderate level of genetic variation in Selenium (Se), Zinc (Zn) and Iron (Fe) accumulation (Thavarajah et al. 2010). Therefore, it is important to determine the genetic control of accumulation of these

micronutrients in pea.

The hypotheses tested in this research were that genomic regions associated with mycosphaerella blight resistance, lodging resistance and micronutrient concentration will be found in the pea cultivars Carrera and CDC Striker and these will be located by QTL mapping the Carrera/CDC Striker recombinant inbred line population.

The objectives of this study were to determine the genetic control of mycosphaerella blight, lodging, and micronutrient accumulation in field pea by genotyping and phenotyping a recombinant inbred line population segregating for these traits and to identify associated quantitative trait loci.

2.0 LITERATURE REVIEW

2.1 Field Pea Varieties, Value and Production

2.1.1 Field pea varieties

The major market classes of pea are round seeded and wrinkled seeded. Round-seeded pea, also called dry pea or field pea, is primarily used for food and feed, and is the main type grown in Saskatchewan. Wrinkled-seeded pea, also called garden pea, vegetable pea or vining pea, is typically harvested when seeds are immature, and is used for freezing and canning. Field pea production in western Canada consists of yellow and green cotyledon cultivars (Saskatchewan Pulse Grower, 2002). In terms of nutrient content, there is no major difference between green and yellow cotyledon peas. Minor differences in seed size and hull thickness have been observed between green and yellow peas (Hickling, 2003). Pea seed dry weights commonly range from 150 to 300 g/1000 seeds at maturity.

Pea plants have three kinds of leaf types: normal leaf type, semi-leafless type and complete leafless type. Normal leaf type consists of one or more pairs of opposite leaflets borne on a petiole together with several pairs of tendrils. Leaflets are broad and ovate. Two stipules at the base of the leaf are also ovate and larger than the leaflets. In the semi-leafless leaf type, leaflets are replaced by tendrils but stipules are still present. In the leafless leaf types, leaflets are also replaced by tendrils and stipules are stunted (Frame, 2011). Semi-leafless and leafless leaf types can reduce lodging since the inter-twining nature of the larger tendril mass can allow neighbouring plants to mutually support each other to produce a rigid canopy (McPhee, 2007). Since the leafless types tend to be low yielding due to their much reduced photosynthetic area, semileafless varieties are recommended for production in Saskatchewan (Saskatchewan Pulse Grower, 2002)

2.1.2 Field pea utilization

Field pea is utilized as dry seeds, while garden pea is marketed as a succulent vegetable. Field pea is utilized for human consumption or fed to livestock (Schata and Endres, 2003). Field pea contains higher levels of the amino acids lysine and tryptophan than those in cereal grains. Protein concentration is about 21 - 25 % in field pea. It is rich in complex carbohydrates, phosphorus and calcium, as well as a good source of vitamin A and D (Tekeli and Ates, 2003). In addition, field pea has a much lower percentage of trypsin inhibitors (5 – 20%) than soybean and can be directly fed to livestock without going through the extrusion heating process (Tekeli and Ates, 2003). Field pea is widely used in forage crop mixtures with small grain cereals, and is a beneficial protein supplement in swine, poultry and ruminant rations (Hauggaard-Nielsen and Jensen, 2001). Field pea mixed with oat and barley can yield a greater biomass dry weight per hectare than a straight pea culture. Cutting should start at the end of flowering and early podding to improve forage yield and quality (Jenkins et al. 2005).

Field pea can be used as green manure or a green fallow crop due to its ability to fix atmospheric nitrogen and convert it into a form that is available to subsequent crops, reducing the need for N fertilizer application. Soil quality and subsequent crop productivity is enhanced in the season after field pea production. Utilization of field pea can protect from soil erosion, improve soil quality and reduce water loss by evaporation or transpiration (Lupwayi et al. 2011). Also, weeds, disease and insect problems can be reduced by including pulse crops like pea into traditional cereal-based crop rotations (Bailey et al. 2001), so field pea fits well into crop rotation systems in western Canada.

2.1.3 Field pea production

Canada, France, and Russia are the top three countries producing field pea, followed by India, the United States and Australia (FAOSTAT, 2008; McVicar et al. 2009). The Indian subcontinent (India, Pakistan, Bangladesh) is the most important import region for field peas exported from Canada, taking 46% of the six-year average (2000-2005). Japan is the biggest importer of Canadian marrowfat peas primarily processed into roasted and salted snack foods (Mercantile Consulting, 2005). In the 1990s, the European Union was a consistent importer of Canadian peas for use as livestock feed, primarily for swine, importing approximately 700,000 tonnes per year (McVicar et al. 2009). Western Canada is the major market for Canadian feed peas, which are used in a variety of animal feeds. As well, peas are processed by several companies for feed uses in the form of individual ingredients or combined with other ingredients such as canola meal. In the past five years, feed markets have become less important as the demand from India for food peas has increased the price of peas, making them generally too expensive for feed markets (Warkentin, personal communication).

Canada, Europe, Australia, and the United States are the main exporters of peas. Currently, Canada is the largest pea producer and exporter in the world and pea production has risen in Canada over the past two decades.

Pea was grown on over 1.24 million hectares in Saskatchewan in 2008, in comparison to only 300 hectares in 1967. The 10-year average pea yield in Saskatchewan is approximately two tonnes/ha. In terms of increasing the diversity of crop production, pea has been a leading alternative crop in Saskatchewan (McVicar et al. 2009).

2.2 Mycosphaerella blight

2.2.1 Life cycle of *Mycosphaerella pinodes*

Mycosphaerella blight, the most important fungal pathogen affecting field pea (*Pisum sativum* L.) in western Canada, is caused by *Mycosphaerella pinodes* (Berk. and Blox.) Vestergren. (Zhang et al. 2007; Xue, 2000). This disease typically appears around flowering time and may spread rapidly (Tivoli et al. 1996). It causes 10% average annual yield loss and more than 50% in individual trials (Xue et al. 1996; Zhang et al. 2004). Mycosphaerella blight is a polycyclic disease. The primary sources of inoculum are airborne ascospores and pycnidiospores which can be splashed by rain or released at early stages of pea growth from former pea residue (Zhang et al. 2005). *M. pinodes* is a semi-saprophytic pathogen which can survive over winter on pea residue. It can survive more than 12 months at soil temperatures ranging from -20 to 30 ℃ by forming chlamydospores with thick walls (Wallen and Jeun, 1968). Ascospores can cause uniform disease over large areas since they can be transmitted by wind more than 1 km from a source. Pycnidiospores which cause secondary infections of *M. pinodes* are distributed more locally (Lawyer, 1984; Zhang et al. 2004).

2.2.2 Symptoms and severity of mycosphaerella blight on pea

Small brown to black lesions appear on leaves after being infected by *M. pinodes*, remaining small under dry condition, but can enlarge under moist conditions (Kraft et al. 1998). Before lesions enlarge, leaves with lesions become dry, particularly on the lower parts of pea plants. Stem lesions appear at the bottom of the dead leaf and spread above (Tivoli et al. 1996). Affected leaves may be killed and stems totally girdled, causing a plant with blue-black appearance and reduced height (Timmerman-Vaughan et al. 2004). Pods infected at this time appear necrotic and become distorted (Tivoli et al. 1996). Seeds can be infected due to pod disease, resulting in seed staining, seed quality reduction and subsequent loss of economic value (Timmerman-Vaughan et al.

2004). The pathogen can survive in infested stubble for several years (Xue et al. 1996) and infect subsequent crops. It can also be dispersed over long distances through the dissemination of air-borne spores (Tivoli et al. 1996).

Any part of the pea plant at any age can be infected by this fungus, especially mature leaves (Kraft et al. 1998). The morphological and physiological characteristics of the plants as well as environmental conditions can strongly affect the plants' reaction to mycosphaerella blight (Worth, 1999). Pea leaf tissue can produce pisatin, which decreases in concentration when the leaves senesce, resulting in the decline of this phytoalexin. This might be the reason for the increasing susceptibility to mycosphaerella blight of the lower parts and senescing leaves of pea (Kraft et al. 1998). As well, the higher disease scores in the lower parts of the pea plant may result from the higher humidity in the canopy at the base of the plants (Tivoli et al. 1996).

Under moist conditions, mycosphaerella blight can spread from plant to plant and mature leaves are most susceptible. Banniza et al. (2005) reported that precipitation or irrigation can increase the severity of mycosphaerella blight ratings from 6.5 to 8.8 on whole plants at physiological maturity, from 1 to 4 on pods, and from 5 to 8 on stems (scale 0-9, 0 = no disease; 9 = whole plant severely blighted). Zhang et al (2005) also indicated that humidity is a major factor influencing pycnidia formation in western Canada. Infections before the mid-flowering stage caused a greater effect on yield and quality than at later stages (Xue et al. 1997).

2.2.3 Strategies for managing mycosphaerella blight

Management practices which may prevent losses caused by *M. pinodes* include the following: (1) cultural practices, (2) chemical control such as seed treatment, foliar sprays and soil treatment.

2.2.3.1 Cultural practices

The aim of cultural control is to reduce the amount of primary inoculum such as infected seeds, infected pea stubble and soil-borne inoculum. Seed-borne inoculum can be reduced by planting seed free of ascochyta blight (Moore, 1946). In addition, it is good to plant crops destined for seed production in areas of lower rainfall whenever possible to reduce the risk of seed being infected by ascochyta blight (Lawyer, 1984). Utilizing seed from crops with least disease is another strategy for raising high quality seed (Wade, 1951). Level of seed infection can be tested and determined before use. For example, seed lots having more than 10% seed infection should not be utilized for seed. Gadd (1950) reported that the level of seed-borne inoculum can be reduced by immersing it in hot water for a short period without seed damage. However, this may not be practical when dealing with large quantities of seed.

Infected pea stubble is another source of primary inoculum (Bretag and Ramsey, 2001). Crop rotation and the burial and destruction of infected pea stubble before the new crop emerges are necessary, since it is difficult to remove all of the infected pea stubble (Lawyer, 1984). The use of 3- to 4-year rotations and growing of non-host crops between pea crops can minimize losses caused by infection of ascochyta blight, while the severity of ascochyta blight may rapidly increase with a short interval between pea crops (Bretag et al. 2001).

2.2.3.2 Chemical control

2.2.3.2.1 Seed treatment

Many fungicides have been developed and used to control seed and soil-borne pathogens (Ogle, 1997). Fungicides based on copper and mercury were initially applied to seeds (Walker, 1961). These fungicides were applied to deal with soil-borne pathogens causing damping-off and some seed-borne diseases including ascochyta

blight (Grewal et al. 1981). Seed-borne fungi on the seed coat were killed after seed treatment with those fungicides, while the soil-borne fungi populations were reduced, (Hassan and Cox, 1947). Copper and mercury were more effective at controlling surface-borne fungi than with fungi under the seed coat and within the cotyledons. Additionally, fungicides containing mercury have been banned in most countries in recent years due to their high toxicity to animals and humans (Bretag et al. 2006). Fungicides such as Apron Maxx RTA® and Vitaflo 280® can provide protection against both seed- and soil borne mycosphaerella blight on pea after seed treatment (Saskatchewan Agriculture's Guide to Crop Protection, 2010).

2.2.3.2.2 Foliar sprays

Foliar sprays of fungicides can effectively control ascochyta blight (Bretag et al. 2006). Broad-spectrum organic fungicides such as mancozeb (Warkentin et al. 2000), thiram (Cass Smith, 1954) and chlorothalonil (Warkentin et al. 1995) have been used successfully to control ascochyta blight. Foliar fungicides including Bravo 500®, Headline EC®, and Quadris® are registered for use on field pea (Saskatchewan Ministry of Agriculture, 2009). However, more than one application of fungicides were usually required for total control and large quantities of water were required for uniform coverage (Bretag et al. 2006).

Warkentin et al. (1995) reported that application of chlorothalonil or benomyl fungicides were effective in reducing the disease severity and increasing field pea yield in small plots, but repeated applications of fungicides were not economically sound due to the cost of the fungicides. In order to decrease production costs and better preserve the environment, the most effective way to control this disease is to develop resistant pea cultivars that can be widely used in commercial production (Xue et al. 1997). However, breeding resistant pea varieties to *M. pinodes* is difficult due to the

availability of only partial resistance (Prioul et al. 2003), the polygenic inheritance of the resistance (Timmerman-Vaughan et al. 2002), and the difficulty of evaluating resistance under field conditions because of interaction between genotypes and environments (Xue et al. 1997; Wroth and Khan, 1999).

2.2.4 Mycosphaerella blight resistance genes in pea

Pea cultivars with complete resistance to the mycosphaerella blight pathogen have not been developed (Timmerman-Vaughan et al. 2002), but sources of partial resistance have been identified in pea lines (Prioul et al. 2004). Clulow et al. (1991b) reported qualitative inheritance of mycosphaerella blight resistance, however, Timmerman-Vaughan et al. (2002, 2004), Tar'an et al. (2003) and others indicated that inheritance of resistance was quantitative. Clulow et al. (1991b) found two dominant genes (*Rmp1* and *Rmp2*) for stem resistance, and another two dominant genes (*Rmp3* and *Rmp4*) for resistance at the seedling stage. Timmerman-Vaughan et al. (2002, 2004) developed a QTL map, identifying six QTLs associated with genomic regions in pea for resistance to ascochyta blight. Tar'an et al. (2003) found three QTLs associated with resistance to *M. pinodes* under field conditions. Prioul et al. (2004) identified six QTLs associated with resistance to *M. pinodes* at the seedling stage under controlled conditions and ten QTLs associated with resistance at the adult stage. Four of these QTLs were common to both stages under both conditions. Prioul-Gervais et al. (2007) mapped resistance gene analogs (RGA) and defense-related (DR) genes, identified QTLs for resistance to *M. pinodes* and co-located candidate genes with QTLs. Fondevilla et al. (2008) identified six QTLs associated with resistance to *M. pinodes* under either growth chamber or field conditions.

2.3 Lodging

2.3.1 Severity of lodging

Lodging is a phenomenon in crops whose stems bend over close to the soil surface, resulting in the collapse of the canopy (Holland, 1990; Amelin and Parakhin, 2003). During the stages of plant development, lodging is affected by environmental factors including wind and rain (Holland, 1990). Lodging prior to pod filling often results in only partial fruit or seed development. Lodging at maturity could make pods inaccessible to harvesters. Lodged plants are typically exposed to increased disease infection due to increased humidity (Acquaah, 2007). Lodging can result in up to 74% yield loss in field pea because it can increase the severity of fungal disease by increasing the humidity in the canopy microclimate, decreasing the crop's photosynthetic rate and increasing harvest cost (Amelin and Parakhin, 2003).

2.3.2 Traits which improve lodging resistance

Pea plants with upright growth are easier to harvest and have lower yield losses. Upright growth improves air circulation in the canopy to reduce disease development (Wang, 1998). Upright growth is enhanced by the semi-leafless leaf type in which tendrils are substituted for leaflets. Banniza et al. (2005) indicated that pea cultivars with normal leaf type were more susceptible to lodging than plants with semi-leafless leaf type.

Stem stiffness is one of the desirable traits to increase lodging resistance. The content of fiber and lignin, which provide mechanical support in pea stems, were negatively correlated with lodging score. Lodging resistance increased with a higher proportion of supportive tissue and xylem in the cross-sectional area of the stem (Banniza et al. 2005).

Plant height, depending on the internode number and internode length is a complex trait and is influenced by genotype and environment. Dwarf or semi-dwarf

pea cultivars are characterized by short internodes, whereas tall cultivars usually have long internodes. Dwarf cultivars typically have greater lodging resistance than tall cultivars (Vasileva et al. 1980). Thus, breeding dwarf plants with short internodes while retaining the same internode number is recommended for lodging resistance (Obraztsov and Amelin, 1990).

Lodging resistance is a quantitative trait involving aspects of stem stiffness, plant height and leaf type (Tar'an et al. 2003). Reduced plant height is considered an effective way for reducing or eliminating lodging (Cooper, 1971; Board, 2001). With each 10 cm increase in plant height, there was a 0.3 increase in lodging score (1 = erect; 5 = completely lodged) among a range of genotypes of soybeans (Wilcox and Sediyama, 1981). Cooper (1971) recognized that lodging increased with plant population. Lower plant population was associated with reduced plant height and wider stem diameter which decreased lodging.

Breeding for lodging resistance is challenging for it is a quantitative trait whose expression is significantly influenced by the environment. Factors that result in lodging can occur at different stages in plant development (Acquaah, 2007). However, breeding for pea plant lodging resistance is important in order to increase grain yield and facilitate harvest. Lodging resistance enhances harvest and reduces the severity of mycosphaerella blight (Banniza et al. 2005).

2.4 Micronutrients (Selenium, Zinc and Iron)

2.4.1 Micronutrient malnutrition

Micronutrient malnutrition is an important issue in human health, especially in many developing countries. All age groups can be affected, especially young children and pregnant women (Ramakrishnan, 2002). Inadequate food intake, poor quality diet and poor bioavailability of nutrients are typically the reasons causing this health issue.

Health problems such as child survival, growth, development and morbidity can be influenced by the status of micronutrients (Ramakrishnan, 2002).

Micronutrient malnutrition affects nearly three billion people around the world resulting in learning disabilities among children, increased morbidity and mortality rates, lower worker productivity and high healthcare costs (Allen et al. 2006; Welch and Graham, 2004). Childhood stunting mainly resulted from micronutrient malnutrition from early foetal stage of development to the fourth year of life (Branca and Ferrari, 2002; Allen et al. 2006). Nutritional deficiencies [e.g. selenium (Se), zinc (Zn), and iron (Fe)] directly or indirectly lead to almost two-thirds of the deaths of children around the world. The risk of death from common diseases such as acute gastroenteritis, pneumonia and measles can also be increased by nutritional deficiencies (Caballero, 2002).

Selenium is one of the essential micronutrients which is limiting in the diets of many people in the world particularly in Asia. Se is a necessary element for humans and animals (Bordoni et al. 2008). Low Se intake can result in Keshan, Kaschin-Beck or Urov diseases. Keshan disease is a cardiomyopathy associated with a low selenium intake and low levels of selenium in blood and hair. Cardiac insufficiency and arrhythmias, congestive heart failure and heart enlargement are symptoms of Se deficiency (Ge andYang, 1993). Kaschin-Beck can cause osteoarthropathy, joint problems and growth stunting, most frequently occurring in pre-adolescent and adolescent children. Keshan and Kaschin-Beck are found in parts of China, Siberia, Japan and Korea (Chen et al.1979; Moreno-Reyes et al. 2003).

Zinc deficiency can increase morbidity from common child infections, such as diarrhea, pneumonia and malaria. In addition, stunting could be caused by Zn deficiency, since Zn interacts with various proteins (Umeta et al. 2000; Graham, 2008).

About one third of children in less wealthy regions around the world are affected by growth stunting due to poor quality diets (Allen et al. 2006). Symptoms including detmatitis, retarded growth, diarrhea, mental disturbances and recurrent infections appear due to severe deficiency. It is difficult to diagnose these symptoms because of their diversity and the lack of suitable biomarkers of Zn deficiency which are available so far (Hambidge, 2000).

Iron deficiency is the most widespread nutrient deficiency in the world, affecting almost 25% of the world's population (Caballero, 2002). Anemia, which is defined as low blood haemoglobin, is caused by severe Fe deficiency, is used as an indicator of this deficiency. Physical endurance can be reduced by Fe deficiency even in the absence of anaemia. Risk of both maternal and child mortality is increased by severe anaemia (Ramakrishnan, 2002). Several diseases such as malaria, genetic abnormalities and chronic disease can be caused by Fe deficiency (Allen and Casterline-Sabel, 2001).

2.4.2 Importance of micronutrients for health

Selenium is also an important component of various enzymes involved in immune functions and is known as the 'King of cancer' among trace elements in humans. Se plays a vital role in prevention and treatment of diseases, promotion of health and prevention of aging (Zheng et al. 2009). Se is an integral part of glutathione peroxidases and selenoprotein P. It is also vital in cancer prevention, HIV treatment and protection from toxic heavy metals (Bordoni et al. 2008). Various diseases such as arsenicosis in Bangladesh, fatal juvenile cardiomyopathy in China, poor skeletal muscle strength in adults, and chronic heart failure can occur due to low intake of Se (<25 μg day^{-1}). In contrast, primary or secondary diabetes may happen as a result of excessive intake of Se (Hawkes et al. 2004). Thus, 55 μg of Se day^{-1} and 60-75 μg of Se

day^{-1} have been recommended for adults in the United States and the United Kingdom, respectively (Broadley et al. 2006).

Zinc is also an essential component of at least 300 enzymes accelerating cell growth and differentiation in tissues. These enzymes are involved in including mitosis, DNA and protein synthesis, gene expression and activation. Zn is also beneficial for enhancing immune systems (Osendarp et al. 2003). Zn plays an important role during periods of rapid growth and development, particularly periods of gestation and fetal growth (McCall et al. 2000). It is recommended that pregnant women especially during the last two trimesters absorb 3 mg Zn/day. Assuming bioavailability is 20%, an intake of 15 mg Zn/day is necessary (WHO, 2002). Intake of 8-14 mg Zn/day by pregnant women was observed in developed countries (Caulfield et al. 1998), whereas an intake of 6– 7 was reported in studies in Malawi (Ferguson et al. 1995) and Brazil (Lehtil. 1989).

Iron is an important component of various enzymes and many proteins, such as cytochromes, involved in vital processes including oxidative metabolism, which is essential for human food supply. Most of the Fe is present as haemoglobin in the human body responsible for carrying oxygen from the lungs to the tissues. It is stored in the liver as ferritin and as haemosiderin (Allen et al. 2006; Prasad and Nirupa, 2007). Daily consumption of 5 to 20 mg iron in a fortified sauce was found to be an effective strategy in the treatment of Fe-deficiency anemia in children (Mannar and Boy Gallego, 2002).

2.4.3 Micronutrient availability internationally

Selenium concentration in soil differs in distribution and chemical availability. The average Se concentration in soils around the world is 0.1 – 2.0 μg of Se kg^{-1}. Plants grown in New Zealand, Australia, Denmark, some regions in China, India and

Bangladesh are low in Se due to its deficiency in the local soils, whereas soil is rich in Se in Colombia, Ireland, and the plains of the United States and Canada (Berrow and Ure, 1989; Combs, 2001).

Soils in Saskatchewan are rich in Se. Total soil concentration from major soil zones in eight different locations in Saskatchewan ranged from 37 to 301 μg of Se kg^{-1}, which may have the potential to provide a significant natural source of this essential element to the plants grown in Saskatchewan (Thavarajah et al. 2008). Canadian field peas are rich in Se whose average total concentration was 0.331 mg kg^{-1}, while large regions of Asia and Europe have soils deficient in Se (Gawalko et al. 2009), thus a good opportunity is available to market Canadian peas to these regions for nutritional benefits. Thavarajah et al. (2007) suggested that conventional plant breeding methods or optimum agricultural production conditions may change the concentration and the chemical forms of Se in pulse crop seeds.

Zinc deficiency is found in many countries and regions in the world including Sub-Saharan Africa, South Asia, East and West Asia and Europe (Alloway et al. 2008). Zn deficiency arises from deficiency in crops planted in these areas. Zn deficiency ranged from less than 10% in Lebanon to more than 80 % in Iran mainly due to high concentration of $CaCO_3$ in soils, giving rise to salinity or sodicity problems (Malakouti, 2007).

Iron deficiency is one of the main deficiency disorders across Europe (Hallberg, 1995). Twenty percent of teenage girls are Fe-deficient across Europe (Hercberg et al. 2001). Forty-eight percent of infants under 2 years of age were affected by Fe depletion (Hercberg et al. 2001). Fifty-seven percent of children under 4 years of age had Fe intakes below recommended levels, leading to 20% of pre-school children having low Fe stores and 8% with Fe deficiency anemia (Gregory and Lowe, 1995).

2.4.4 Biofortification

Low concentrations and low availabilities of micronutrients in daily diets are the main reasons for micronutrient deficiencies in humans. Supplementation of products and the fortification of food with micronutrients have been used to address the issue of deficiencies. However, these approaches have not been ideal due to their high cost and low coverage, even though they are effective in dealing with severely deficient people in some cases. A new approach called biofortification is a sustainable means to address the problem of micronutrient deficiencies by increasing the density and bioavailability of micronutrients in the edible parts of plants (Bouis and Welch, 2010; Welch and Graham, 2004; Yang et al. 2007).

This strategy is a feasible means of targeting the population that have limited access to markets or healthcare facilities that provide fortified foods and nutritional supplements. Biofortification can reduce the number of people with malnutrition. This approach is also relatively low cost and highly efficient and it also provides wide coverage, especially in developing countries (Bouis and Welch, 2010).

2.5 DNA Markers

A DNA marker is a DNA segment whose sequence is readily detected and inheritance can be easily monitored (Kumar et al. 2009). DNA markers are widely used because of their abundance. DNA markers play an important role in assisting global food production by enhancing the efficiency of selection for traits of interest. They come from different kinds of DNA mutations such as substitutions (point mutations), rearrangements (insertions or deletions) or errors in replication of DNA. DNA markers are usually located in non-coding regions, therefore, they are selectively neutral, not affecting the phenotype of the trait of interest (Collard et al. 2005). There are different types of DNA markers such as PCR based markers (including RAPD, AFLP, SSR, and SNP) and non-PCR based markers (including RFLP) (Kumar et al. 2009).

2.5.1 RFLP markers

Restriction fragment length polymorphism (RFLP) is a technology that can differentiate among organisms by analysis of fragments derived from cleavage of their DNA. RFLPs have moderate polymorphism, high genomic abundance, random distribution and high reproducibility (Kumar et al. 2009). They are codominant markers and no prior sequence information is needed. They can differentiate whether a linked trait is in a homozygous or heterozygous state (Semagn et al. 2006). However, RFLPs are expensive since they require the use of radioactive probes. The assays are time-consuming and laborious, for large quantities and high quality of DNA are needed (Paran and Michelmore, 1992).

2.5.2 AFLP markers

Amplified fragment length polymorphism (AFLP), which depends on the selective PCR amplification of restriction fragments, is a whole-genome fingerprinting method (Semagn et al. 2006). Even though AFLP is a dominant marker that cannot distinguish between homozygous and heterozygous individuals, it is powerful for producing reliable banding without probe development. It does not require any prior DNA sequence information from the organism under study and it has the ability to analyze a large number of polymorphic loci simultaneously (Semagn et al. 2006). As well, AFLP analysis requires very small quantities of DNA. Furthermore, the DNA template produced can be used for hundreds of PCR amplifications (Kumar et al. 2009).

2.5.2 SSR markers

Since simple sequence repeats (SSRs, also known as microsatellites) are short sequences of 1-6 nucleotides repeated in tandem. They are single-locus and multi-allelic markers (Hwang et al. 2009). SSRs are inherited in a codominant manner at individual loci, so that homozygous and heterozygous plants can be distinguished.

As well, they are distributed equally and randomly in the genome (Akkaya et al. 1995; Hwang et al. 2009**)**. With the use of multiple PCR analysis, it is fast and cost-effective to genotype SSR markers by simultaneous detection of multiple alleles at one locus (Tang et al. 2003). Thus, it is easy to analyze SSRs through PCR when flanking unique sequence primers are used (Taramino and Tingey, 1996).

2.5.4 SNP markers

Single nucleotide polymorphism (SNP) is a codominant marker type which has recently become popular in genomic studies. The polymorphism comes from a change in a single nucleotide position (point mutations). Sequence information is required in the analytical procedures to design the allele-specific PCR primers or oligonucleotide probes. Library construction and sequencing can be used to find SNPs and flanking sequences. One of the merits is the possibility of high throughout automation, once the location of SNPs is identified and appropriate primers are designed (Kumar et al. 2009). In addition, the SNPs are abundant throughout the genome in various species including plants. SNP marker system is attractive for mapping and marker-assisted breeding due to the abundance of these polymorphisms in plant genomes (Semagn et al. 2006). It can be used to understand the genomic variability and diversity between the same and different species (Kumar etal. 2009). SNP genotyping is based on allelic discrimination rather than based on size differences on a gel (Semagn et al. 2006).

2.6 QTL Mapping

2.6.1 QTL mapping methods

Positions and relative genetic distances between markers along chromosomes are referred to as linkage maps. Quantitative trait loci associated with traits of interest in linkage maps are called QTL maps (Collard et al. 2005). Variation in the trait of interest in a specific region of the genome is identified by QTL mapping. Molecular markers

associated with QTL can be effective for marker-assisted plant breeding (Timmerman-Vaughan at al. 2005). Markers are used to partition a mapping population into different genotypic groups in terms of the presence or absence of a particular marker locus (Young, 1996). Several methods have been reported to identify QTL regions based on marker linkage including simple marker analysis, interval mapping and multiple QTL mapping (Jansen, 1993; Lander and Botstein 1989).

2.6.1.1 Single marker analysis (SMA)

Analysis of variance (ANOVA, sometimes called "marker regression") at the marker loci is the simplest method for QTL mapping (Soller and Brody, 1976). This is a traditional approach that splits progeny into two groups with marker genotype and compares the average of the two marker genotype groups based on t-statistic. If there is no difference between the means of phenotypic effect in two groups, this marker does not appear to be linked to a QTL (Soller and Brody, 1976; Broman, 2001). The main advantages of this analysis are characterized with its simplicity and a genetic map for the markers is not required. However, estimate of QTL location and QTL effect are not obtained. QTL location is only indicated by checking the greatest difference between genotype group averages caused by certain markers. QTL effect at a specific marker will be smaller than the true one in terms of recombination between the marker and the QTL. In addition, missing data in genotypes should be removed and the power for QTL will be decreased if the QTL is located far from all markers (Lander and Botstein, 1989; Broman, 2001).

2.6.1.2 Interval Mapping (IM)

Interval mapping was a widely applied approach for QTL mapping (Jansen and Stam, 1994). Interval mapping usually maps one QTL at a time, ignoring the effects of other QTLs (Lander and Botstein, 1989). It has several advantages over

analysis of variance at the marker loci. First, it provides a curve indicating the evidence for QTL location at various points along the entire genome. Second, the position of the QTL is given by support intervals and it allows for the inference of QTLs to positions between markers. Third, compared to SMA, it provides improved estimates of QTL effects. As well, it allows incomplete marker genotype data (Broman, 2001; Jansen and Stam, 1994; Lander and Botstein, 1989). However, the approach of IM considers one QTL at a time in the model for QTL mapping and thus, IM can miss the identification and estimation of QTL when multiple QTLs are located in the same linkage group (Lander and Botstein, 1989). In comparison to SMA, IM requires specially designed software, increasing computation time (Broman, 2001).

2.6.1.3 Multiple QTL mapping

Jansen (1993) and Zeng (1993) independently advised combining IM with regression analysis in mapping to deal with multiple QTL problems. This combination was named composite interval mapping (CIM) (Zeng, 1993). By using CIM, other markers can be used as covariates to control for other QTLs when testing for the putative QTL in an interval and can also reduce the residual variance to improve the test (Kao et al. 1999).

Ideally, current QTL mapping models can be extended to a multiple QTL model for mapping multiple QTLs. This QTL mapping method called multiple interval mapping (MIM) which can use multiple marker intervals to simultaneously construct multiple putative QTLs in the model for QTL mapping (Kao et al. 1999). Compared to IM and CIM, MIM tends to be more powerful and precise in detecting QTLs, better in separating linked QTLs and has a greater ability to estimate interactions between QTLs (Broman, 2001; Mackay, 1996). In addition, MIM can analyze epistasis of QTL and estimate the individual genotypic value, the heritability of quantitative traits and

genetic variance components contributed by an individual QTL. Hence, marker-assisted selection can be performed under MIM (Kao et al. 1999).

2.6.2 QTLs in pea

2.6.2.1 QTL mapping of mycosphaerella blight resistance

Resistance to mycosphaerella blight is multigenic and quantitatively inherited (Roger et al. 1999a; Hwang et al. 2006). Disease resistance in pea is one of the traits that has been examined by QTL mapping (Pilet-Nayel et al. 2002).

Timmerman-Vaughan et al. (2002) developed a QTL map based on the population from a cross between 3148-A88 (resistant) and Rovar (susceptible) using RAPD, RFLP, AFLP and SCAR markers. Ascochyta blight resistance of progeny lines was examined over three field seasons. QTLs associated with ascochyta blight were detected on linkage groups (LGs) I, II, III, IV, V, VII and group A. The location of the QTL on LG III remained unknown and was designated *Asc3.1*. Two QTLs in LG IV were designated as *Asc 4.1* and *Asc 4.2*. QTLs (*Asc 1.1*, *Asc 2.1*) located on LG I, II, respectively. *Asc 5.2* located on LG V and linked to SAFP2P2c. These resistance QTLs explained 8-35% of the phenotypic variation individually.

Tar'an et al. (2003) identified QTLs on LGII (*ccta2*), LG IV (*cccc1*) and LG VI (*acct1*) associated with reaction to mycosphaerella blight by using AFLP, RAPD and STS markers. Altogether, these three QTLs accounted for 35.9% of the phenotypic variation for the reaction to mycosphaerella blight. The QTL at *acct1* locus was also identified to be associated with lodging resistance. Lodging was positively correlated with reaction to mycosphaerella blight ($r=0.35$, $P<0.01$), meaning genotypes with increased resistance to the disease might be less prone to lodging (Tar'an et al. 2003).

Prioul et al. (2004) mapped and characterized QTLs for seedling- and adult-stage resistance to *M. pinodes* in pea using a RIL population derived from the cross between

DP (partially resistant) and JI296 (susceptible) by using RAPD, SSR and STS markers. The major part of the variation observed for seedling resistance was identified on both stipules and stems. Six QTLs localized on linkage groups (LGs) III, Va, VI and VII were found, which explained from 5% to 20% of the total phenotypic variation. The QTLs altogether accounted for 73% and 74% of the total variance on stipules and stems, respectively. Five QTLs were common to both stipules and stems except *mpIII-3* that was specific to stems and explained only 6% of phenotypic variation. *mpIII-1* and *mpVI-1* explained a major part of the variation, while other QTLs showed minor effects. All of these alleles for resistance to *M. pinodes* were derived from the resistant parent DP, except for the *mpIII-2* located on the distal part of the linkage group III which was derived from susceptible cultivar JI296. At the adult stage, ten QTLs distributed on LGs II, III, Va and VII for partial resistance to *M. pinodes* were identified in the field. Each QTL explained 6% to 42% of the total phenotypic variation. Altogether, QTLs accounted for 63.7% and 56.6% of the total phenotypic variation on the stipules and stems, respectively (Prioul et al. 2004).

Prioul-Gervais et al. (2007) mapped candidate resistance gene analogs (RGAs) on the JI296 X DP genetic linkage map and compared their genomic localizations with QTLs for resistance to *M. pinodes* using RGA primer pairs (second-generation primers: *RGA1a, RGA1b, RGA2, RGA3, RGA4* and *RGA5*; cloned pea RGAs: *RGA-G3A, RGA1.1, RGA2.65 and RGA 2.97*). RGAs are the pea sequences that are analogous to the conserved nucleotide binding site (NBS) domain found in a number of plant disease resistance genes (R-genes) (Timmerman-Vaughan et al. 2000). After comparing polymorphism, one of the RGA loci was mapped on LG VII. Two specific primers were designed in the region of the class II (*IJB174* and *IJB91* sequences) and generated sequences showing polymorphism between the two parents were mapped on

linkage group VII. The polymorphic *RGA3* locus was mapped in the proximity of the class II *IJB174* and *IJB91* on LG VII. *RGA-G3A* and *RGA2.97* were mapped on LG VII in the vicinity of the those markers (*RGA3, IJB174* and *IJB91*). *RGA1.1* was mapped on LG III, but its accurate location was unknown. As well, co-localizations between candidate genes and QTLs for resistance to *M. pinodes* were discovered. The first co-localization was identified on LG III between *PsDof1* gene (Elicitor-responsive Dof protein) and QTL *mpIII-1,* explaining 6-42% of phenotypic variation at both seedling and adult stages (Prioul et al. 2004). Thus, *PsDof1* is a good candidate for the QTL *mpIII-1*. The second co-localization was found on LG III between *DRR230-b* (disease resistance response protein 39) and the *QTL mpIII-4,* explaining 29% of the stem resistance (Prioul et al. 2004). Therefore, *DRR230-b* is candidate for this QTL. The third co-localization was discovered on LG VII between a cluster of RGAs (*RGA2/ IJB174*, *RGA3*, *IJB91*, and *RGA-G3A*) and *mpVII-1*, minor-effect QTL in stipule resistance in both seedling and adult stages. Hence, these four RGAs can be considered as valuable candidate genes for the QTL *mpVII-1* (Prioul et al. 2007).

Fondevilla et al. (2008) developed a linkage map using a RIL population derived from a cross between the wild *Pisum sativum* subsp. *syriacum* accession P665 (resistant) and a susceptible pea cultivar (Messire) which was analyzed using morphological, isozyme, RAPD, STS and EST markers. P665 had strong resistance under controlled conditions to five different *M. pinodes* isolates from different countries. Its resistance to *M. pinodes* was effective under field conditions as well (Fondevilla et al. 2005). Six genomic regions were identified along LGs II, III, IV and V which explained individually from 9 to 52% of the phenotypic variation associated with resistance to *M. pinodes*. Altogether these six QTLs accounted from 31 to 75% of the phenotypic variation. Among these, *Mp III.1* and *MpIII.2* were associated with

resistance to *M. pinodes* in both growth chamber and field conditions. QTLs *MpV.1* and *MpII.1* were only associated with resistance in seedlings under growth chamber conditions. QTLs *MpIII.3* and *MpIV.1* were specific for adult plant resistance in the field. QTLs *MpV.1, MpIII.1, MpIII.2* and *MpIII.3* were derived from the resistant parent P665, while the QTLs *MpII.1* and *MpIV.1* originated from the susceptible parent Messire. Knowledge of resistance to *M. pinodes* in wild accessions could accelerate gene transfer to pea cultivars (Fondevilla et al. 2005).

2.6.2.2 QTL mapping of lodging resistance

The dwarfing gene *le* enhances lodging resistance in many field pea cultivars and was located at the far end of LG III in a RAPD genetic map (Rameau et al. 1998). QTLs associated with lodging resistance in pea related to stem stiffness and plant height have been mapped using AFLP and SSR markers (Tar'an et al. 2003). Tar'an et al. (2003) showed that the *cacc4* locus, accounting for 47% of the total phenotypic variation of mean lodging reaction across 10 environments, was strongly associated with lodging resistance and located relatively in the middle of LGIII. In addition, the *acct1* located on LG VI was also identified for lodging reaction, which accounted for 26% of the phenotypic variation (Tar'an et al. 2003).

2.6.2.3 QTL mapping of micronutrient accumulation

No studies on QTL mapping of micronutrient accumulation (Se, Zn and Fe) have been conducted in pea. Several studies identified QTLs associated with micronutrient accumulation (Se, Zn and Fe) in rice (Zheng et al. 2009, Norton et al. 2010) and wheat (Shi et al. 2008, Tiwari et al. 2009).

Therefore, it is possible to identify and map quantitative trait loci that are associated with traits including mycosphaerella blight resistance, lodging resistance and high selenium concentration.

3.0 Materials and Methods

3.1 Plant materials

Carrera and CDC Striker were used as parents in this experiment. Carrera has yellow cotyledons, semi-leafless leaf type, is susceptible to powdery mildew (*Erysiphe pisi* DC.), *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr (Bing et al. 2007) and lodging (Zhang et al. 2006). Carrera was developed by Cebeco Zaden (now Limagrain), the Netherlands, from the cross Belinda/Cebeco 756-921. Both Belinda and Cebeco 756-921 were developed by Cebeco Zaden (Warkentin, personal communication). Medium level of micronutrient (Se, Zn and Fe) accumulation was found in Carrera (Thavarajah, personal communication).

CDC Striker has green cotyledons, semi-leafless leaf type, is susceptible to powdery mildew, and moderately susceptible to *Mycosphaerella pinodes* (Berk. & Blox) Vestergren. CDC Striker was developed from the cross Majoret/P28RS-281 which was made in 1995 at the University of Saskatchewan. Majoret was developed by Svalof-Weibull, Sweden. P28RS-281 is derived from the cross Century *afst*/Maestro made by Spectrum Specialty Seeds Ltd., Saskatoon, SK (Warkentin et al. 2002). CDC Striker has good lodging resistance, medium-sized, round and smooth seeds, and good yield potential. CDC Striker showed medium level of micronutrient (Se, Zn and Fe) accumulation (Thavarajah, personal communication). Both Carrera and CDC Striker are high yielding cultivars which are adapted to the field pea growing regions of western Canada. These cultivars are contrasting in that among pea cultivars grown in western Canada, CDC Striker is rated among the best in terms of resistance to mycosphaerella blight and lodging, while Carrera is rated among the worst for these traits (Warkentin, personal communication).

A population consisting of 142 recombinant inbred lines (RILs) was developed at

the Crop Development Centre, University of Saskatchewan from the cross of Carrera and CDC Striker. Unselected individual plants in F_2 were inbred to the F_7 generation using single-seed descent in the College of Agriculture & Bioresources, University of Saskatchewan greenhouses. A total of 69 RILs have yellow cotyledons, 67 RILs have green cotyledons, and 6 RILs contain both yellow and green seeds; these ratios fit the expectation for the segregation a single gene trait such as cotyledon colour. Seeds from each RIL were bulked and increased in the field in the F_8 generation in summer 2009 in 1 m^2 microplots.

3.2 Field trials

3.2.1 Experimental design

The experimental design was a 12 X 12 lattice (142 RILs plus parents) with 2 replications. This experiment was conducted at 2 locations (Saskatoon and Rosthern) in Saskatchewan, Canada in 2010 and 2011.

Fig. 3.1 A portion of the field trial at Rosthern, SK in 2010 used to evaluate the recombinant inbred line population derived from the cross Carrera/CDC Striker.

Each RIL was planted in a microplot (1m^2), i.e., 75 seeds harvested from each F_8 and F_9 plant were respectively planted in 2010 and 2011 with four rows in a 1.0 X 1.0 m microplot with 0.25 m between rows. Microplots of maple peas were planted as

borders through the middle of each replication at each location to avoid microplots being damaged by tractor application of pesticides. As well, borders surrounding the experiment were set up to reduce edge effects.

3.2.2 Management of field trials

The pea seeds were sown on May 14 and May 18, 2010 and on the same days in 2011 at Rosthern and Saskatoon, respectively. Fertility and cultivation regimes were consistent with the optimum pea production practices for these regions. Kumulus DF, containing 80% sulfur, a contact fungicide registered for control of powdery mildew (Endres, 2004), was sprayed at the first appearance of symptoms (August 5, 2010) at both locations, since all RILs and parents are susceptible to powdery mildew and controlling powdery mildew allowed a clearer evaluation of mycosphaerella blight. Powdery mildew is caused by the fungus *Erisyphe pisi* and symptoms usually first appear in Saskatchewan fields in late July. As powdery mildew appeared only just before physiological maturity in 2011, Kumulus was not applied at either location. Microplots were harvested on September 4, 2010 and September 12, 2011 at Saskatoon and on September 2, 2010 and September 6, 2011 at Rosthern.

3.3 Phenotyping

3.3.1 Mycosphaerella blight

From July to September 2010 and 2011, assessments were made to record mycosphaerella blight severity under natural infection on the basis of an individual microplot using a 0-9 scale where 0 = no disease and 9 = whole plant severely blighted (Xue et al. 1996; Table 3.1). First assessment was made one after flowering, second assessment was made 2 weeks after the first assessment, the third assessment was made when the first RILs reached physiological maturity, and the final assessment was made on the date of physiological maturity for individual RILs. Note that for the earliest maturing RILs, the third and the fourth assessments were the same. Four assessments

of mycosphaerella blight were used to calculate area under the disease progress curve (AUDPC).

Table 3.1. Mycosphaerella blight rating scale used to evaluate the recombinant inbred line population derived from the cross Carrera/CDC Striker according to Xue et al. (1996).

	Plant Position		
Disease severity	Upper	Middle	Lower
0	*F	F	F
1	F	F	L
2	F	F	M
3	F	L	M
4	L	L	M
5	L	M	M
6	L	M	S
7	M	M	S
8	M	S	S
9	S	S	S

Note: * F-free of disease on leaves/stems; L-light infection 1-20% of leaves/stems showing symptoms; M-moderate infection 21-50%; S- severe infection 51-100%

3.3.2 Lodging

Lodging of microplots was assessed on the same date as mycosphaerella blight (See 3.3.1). One additional lodging rating was made 4 weeks after flowering for a total of five ratings. Lodging ratings were recorded using a 1-9 scale where 1 = completely upright and 9 = completely lodged (Wang, 1998; Table 3.2 and Fig. 3.2). Five lodging assessments were used to calculate area under the lodging progress curve (AULPC).

Table 3.2. Lodging rating scale used to evaluate the recombinant inbred line population derived from the cross Carrera/CDC Striker.

Lodging assessing	Lodging development
1	Main stems strictly upright
2	Main stems incline slightly
3	Main stems at 60° angle
4	Main stems at 45° angle
5	Main stems at 30° angle
6	1/2 of the main stems flat
7	2/3 of the main stems flat
8	4/5 of the main stems flat
9	All main stems flat

Fig. 3.2. Examples of lodging variation in pea recombinant inbred lines derived from the cross Carrera/CDC Striker. Left picture was scored as 4; right picture was scored as 7.

3.3.3 Micronutrient concentration

Seed selenium, zinc, and iron concentration were measured as mg kg^{-1} (ppm) in collaboration with Mr. Barry Goetz in the Department of Plant Sciences. Before being digested in a Vulcan 84 (Questron Technology Corporation, Ontario) automatic digestor, ten gram harvested dry field pea seed samples were ground into a fine powder (<0.5 mm sieve). One gram pea powder was weighed and put in a digestion tube (30 ml). In each run, 84 tubes were analyzed in this digestor, among which 4 tubes were blank and 4 tubes were yellow cotyledon lentil (standard). These tubes were placed in the Vulcan 84 and digested in 6 mL nitric acid (HNO_3) for 35 minutes before heating in a heating block at 90 ℃ for 75 minutes. Then 3 mL 30% hydrogen peroxide (H_2O_2) was added and the solution was vortexed for 2 minutes. After approximately 30 minutes, when the bubbling sounds disappeared, 3 mL 6M HCl was added into the vessels. Samples were heated for another 5 minutes and cooled for 45 minutes. The samples were diluted with Millipore water and adjusted to a volume of 25 mL. All procedures conducted by the Vulcan 84 were completed automatically based on the program in the computer. The digestion protocol was based on Thavarajah et al. (2007) with minor modifications.

To determine Se concentration, 9 mL 0.72 M HCl was added to 3 mL digested samples (4X dilution). After preparing one tray, the samples were covered with oven bags and placed in a water bath for 45 minutes at 78 ^{o}C. Then the samples were cooled to room temperature and vortexed. These samples were then analyzed using atomic absorption spectrophotometer (AAS) nova®300 (Analytic Jena AG, Germany).

The concentration of chemical elements by AAS were determined based on the absorption of optical radiation (light) by free atoms in the gaseous state. Each sample concentration was determined by the radiation ratio without a sample and with a sample (the absorbance) (Manual nova®300, 2006). Se concentration in samples was based on the hydride technique. Sodium tetrahydroborate ($NaBH_4$) was used as reactant reacting with acid sample solutions (from weak to strong) and gaseous metal hydrides were formed with the metal ions. The metal hydrides are carried by argon (carrier gas) and by liberated hydrogen to the heated quartz cell until free metal atoms are generated before it exits through the burner slit (Manual nova®300, 2006). The National Institute of Standards and Technology (NIST) standard reference material 1573a (tomato leaves; [Se] = 0.054 $\pm$ 0.003 mg kg^{-1}) was used to measure total Se concentration (Thavarajah et al. 2007).

A total of 5.6 mL of this solution was used to analyze Zn and Fe concentration by AAS following methods similar to Thavarajah et al. (2007). These samples generally do not require any dilution and they are run straight as digested. The sample aerosol is mixed with acetylene and additional oxidant in the mixing chamber before it exits through the burner slit (Manual nova®300, 2006).

3.3.4 Other agronomic traits

Other agronomic traits including days to flowering, days to maturity, plant height and grain yield were measured using the microplot as a unit during the growing season.

Days to flowering was the number of days from the day of sowing to the day when half of the plants in the plot started to flower (Zhang et al. 2006). Light penetration (LP) was measured between 1100 and 1400 h on a day during pod filling stage. A line quantum sensor (Li-Cor LI-191SA, Lincoln, NE), 1 m in length, which measured photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}) firstly measured the light intensity above the pea canopy and then measured twice diagonally in each microplot at the soil surface. Mean values of two measurements at the soil surface were used to calculate LP which was calculated as follows: LP = (Average PAR underneath canopy/PAR above) X 100. Days to maturity was the number of days from the day of sowing to the day when 80% of the pods in the plot have turned yellow (Zhang et al. 2006; Tar'an et al. 2004). Plant height was recorded at the pod setting stage. Grain yield was assessed by combine harvesting the plots and converting to kilograms per hectare. Broken and damaged seeds and foreign material were removed from the samples before weighing. Thousand seed weight was measured after sample cleaning as g/1000 seed. Seed shape and seed dimpling were also measured after seed cleaning. Seed shape was measured based on a 1-5 scale where 1= round and 5= cubed. Seed dimpling (golf ball appearance) was measured based on percentage of seeds with dimpling on seed coat. All of the traits were measured in both 2010 and 2011.

3.4 Genotyping

3.4.1 DNA extraction

Five seeds of each F_9 RIL and parents were planted in pots in the Agriculture greenhouse, University of Saskatchewan in June, 2010. Seeds from the same line were planted in one pot. One month after planting, young fresh leaves were randomly sampled from five plants per line for genomic DNA extraction. DNA was extracted by the hexadecyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al.

1984) and DNA stocks were used in polymerase chain reaction (PCR) amplification.

Procedures of DNA extraction following the CTAB method (Saghai-Maroof et al. 1984) were as follows: 1) 0.2-0.3 g leaf tissue from each plant was placed in a 2 ml Eppendorf tube and covered by a piece of foil with one or a few tiny holes; 2) the tubes with the leaf tissue were quickly put into liquid nitrogen and then put into a vacuum-freeze dryer for 48 hours to dry the samples; 3) the leaf tissue was ground into a fine powder using a high-speed shaker with a tungsten steel bead in each tube; 4) 400 µl of 2×CTAB buffer with newly mixed 1% mercaptoethanol was added into each tube and mixed well with the leaf tissue; 5) the tubes with the samples were incubated for 30 minutes at 65 ℃ in a water bath and inverted 3 times while in the water bath; 6) the tubes were incubated for 10 minutes to return to room temperature; 7) 400 µl of chloroform: isoamyl-ethanol (24:1) was added to each tube and the tubes were shaken continuously for 5 minutes; 8) each tube was centrifuged at 3750 RPM for 10 minutes and 200-300 µl of the upper (aqueous) phase was removed into a clean tube; 9) steps 7 and 8 were repeated once more; 10) 400 µl of –20 ℃ isopropanol (95%) was added into the aqueous phase and the tubes were placed into –20 ℃ freezer for 30 minutes or overnight until DNA precipitated; 11) DNA was spun down and isopropanol was decanted; 12) 400 µl 76% ethanol/0.2M Na-acetate was added to each tube and tubes were incubated for 20 minutes; 13) ethanol/Na-acetate was decanted and 200 µl 76% ethanol/10 mM NH_4-acetate was added to each tube and left for 5 minutes; 14) the tubes were spun for 3 minutes and ethanol/NH_4-acetate was decanted; 15) the tubes were placed upside down on tissue paper for 10 minutes; 16) 200 µl TE buffer was added to each tube and left overnight to allow the DNA to go into solution; 16) the solution was centrifuged 10 minutes to pellet any undissolved particles and the supernatant was transferred to a fresh tube; 17) 5 µl of the DNA solution was diluted

with 95 µl of TE buffer into a UV plate and assayed on a spectrophotometer to quantify the DNA concentration. After DNA quantification, the tubes containing DNA were labeled and stored at -20 ℃ as stocks. A different amount of stock DNA from each tube was taken and diluted with sterilized distilled water to a final volume of 500 µl at a concentration 20 ng/µl. The diluted solution was stored at -20 ℃ and used for PCR amplification.

3.4.2 DNA quality confirmation

DNA quality was tested on agarose gels as follows: 1) 1% solution of agarose gel was prepared by melting 1 g of agarose in 100 ml of 1X TBE buffer by microwave for 2 minutes; 2) 1.5 µl of ethidium bromide was then added after the gel cooled; 3) the gel was cast using tray and comb and allowed to set for a minimum of 20 minutes at room temperature; 4) 7 µl 10 kb ladder and 5 µl samples + 2 µl loading buffer were loaded into separate wells; 5) the gel was run for 30 minutes at 90 V; 6) the gel was exposed to UV light and photographed (demonstration); 6) DNA quality was confirmed by the presence of a highly resolved high molecular weight band indicating good quality DNA (presence of a smeared band indicating DNA degradation) (Vinod, 2004).

3.4.3 SSR analysis

A total of 330 primer sequences for pea SSRs developed by the Agrogene consortium (Agrogene SA, 620 Ave Blaise Pascal, ZI, Moissy Cramayel 77555, France) were used for the genotyping of this pea population. In this study, PSMPS at the beginning of the name of each marker was dropped. In order to facilitate multiplexing possibilities, forward SSR markers with extra universal M13 primers (5'-CACGACGTTGTAAAACGAC-3') on the 5' end were utilized according to Ubayasena et al. (2010) to test this pea population. These M13 attached forward primers along with respective reverse primers and fluorescently labeled (6-FAM, NED,

VIC and PET) M13 universal primer sequence was used in PCR amplification. PCR was performed as reported in Ubayasena et al. (2010) in a total volume of 20 µl containing 10 mmol/L Tris-Hcl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTP, 1 U of Taq polymerase (GeneScript Corp), 0.02 µmol/L M13 attached forward SSR primer, 0.2 µmol/L reverse SSR primer, 0.18 µmol/L FAM-, NED-, VIC-, PET-labeled M13 primer, and 30 ng of DNA. PCR amplification reactions were performed in a PTC-200 thermal cycler using the following thermal protocol: 95 ℃ for 3 min; 4 cycles of touch-down profile consisting of 4 cycles of 94 ℃ for 30 s, 56 ℃ to 50 ℃ (–2 ℃ /cycle) for 50 s, and 72 ℃ for 50 s; and then 25 cycles of 94 ℃ for 30 s, 51 ℃ for 50 s, and 72 ℃ for 50 s. A final extension of PCR products at 72 ℃ for 10 min was allowed before the final step at 8 ℃. After PCR amplification, the plate containing PCR product was labeled and stored at -20 ℃.

Polymorphisms of primers from SSR markers were detected based on parents using polyacrylamide gel electrophoresis (PAGE) after PCR reaction. Twenty µl of loading dye was added to each well in a PCR plate and the PCR plate was placed in the PCR machine at 95 ℃ for denaturing 10 minutes. Three µl PCR products were loaded into PAGE after denaturing. Gels were run about 1.5 hours at 55 ℃ and 75W. The molecules in the gel were stained (2g silver nitrate and 3ml of 37% formaldehyde in 2L of ultra pure water) and developed (6ml of 37% formaldehyde, 800 µl sodium thiosulfate 10mg/ml and 120g sodium carbonate to 4L ultra pure water) to make them visible. Bands were screened based on the results after developing. Only polymorphic primers between parents were selected and applied to RILs.

During the testing of these polymorphic markers on RILs, 1.5 µl of individual PCR products were added to 3.5 µl Hi-Di formamide and 0.075 µl GeneScan 500 LIZ size standard (35 – 500 nucleotides) in each well and run on a 3730 DNA Analyzer (ABI) at

the Plant Biotechnology Institute, National Research Council of Canada in collaboration with Dr. Andrew Sharpe. After running ABI, scoring of each line was conducted using Genographer 2.1 (Benham et al. 1999).

3.5 Statistical analysis

3.5.1 Phenotypic data analysis

Analysis of variance for all the phenotypic traits for each location in each year and combined analysis across locations was performed using the R (http://www.R-project.org/) Mixed-Effects Models. Phenotypic data included individual assessments of mycosphaerella blight and lodging, AUDPC for mycosphaerella blight, AULPC for lodging, micronutrient concentration, days to flowering, days to maturity, plant stand, light penetration, plant height, seed yield, thousand seed weight, seed shape and seed dimpling.

In the analysis of variance (ANOVA), all phenotypic data were considered as response variables. Genotypes, locations and years were considered to be fixed effects and incomplete blocks nested within replications as well as replications were considered as random effects. Normality of distribution ($P>0.05$) was checked using the Shapiro and Wilk test (Shapiro and Wilk, 1965). Mixed model formula **lme** and **lmer** were used in this model in R 2.11 package. Fligner.test (Conover et al. 1981), patterns in residual vs. fitted plots and distribution in residuals were applied to test the homogeneity of variances. Least square means of each trait calculated using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA) across replications, locations and years were used for the estimation of means for the two parents and RILs. Correlation coefficients among the agronomic traits were estimated by using Pearson's product-moment correlation test in R package 2.11. In frequency distribution graphs, values of least significant difference at the 5% level were used to determine whether

there were significant differences between Carrera, CDC Striker, and the RILs.

The genotypes, locations, years, replications and their interactions were considered as random effects to estimate the variance component. Variance components were estimated using **lmer** in mixed model in R package 2.11. The phenotypic variance was estimated as $\sigma^2_p = \sigma^2_g + (\sigma^2_{gy}/y) + (\sigma^2_{gl}/l) + (\sigma^2_{gyl}/ly) + (\sigma^2_e/lyr)$, where σ^2_g is the estimated genotypic variance, σ^2_{gy} is the genotype × year interaction variance, σ^2_{gl} is the genotype × location interaction variance, σ^2_{gyl} is the genotype × year × location interaction variance, σ^2_e is the error variance, y is the number of years tested, l is the number of locations, and r is the number of replicates per location. Broad-sense heritability estimates for each trait were calculated as $H = \sigma^2_g/\sigma^2_p$. The parental cultivars were removed from the data sets to facilitate the estimation of variance components of RILs and QTL mapping analysis.

3.5.2 Genotypic data analysis

Segregation of each polymorphic marker in the RIL population was analyzed for goodness of fit to an expected allelic 1:1 ratio using a Chi-square test (Strickberger, 1985) at P>0.05 in R 2.11. Linkage groups of the markers were determined using Carthagene 1.2.2 (De Keyser et al. 2010) at a LOD (logarithm of odds ratio) score of 3.0 with a maximum distance between two markers of 30 cM (Kosambi). The QTL location and effect for each trait were estimated by composite interval mapping (CIM) using Qgene 4.0 (Nelson, 1997) based on the least square means of phenotypic data from each of the 2 years and over both years. The LOD threshold to determine significant association of the genomic regions with trait was determined by 1000 permutations. The co-factors were specified as five loci identified by stepwise regression that explained the most variation for a given trait.

A single factor ANOVA (R 2.11 package) was performed to examine the association of unlinked markers with phenotypic traits in the population. A significant

association between a marker and a phenotypic trait was declared if the probability was equal to or less than 0.05. The proportion of phenotypic variation accounted for by each detected QTL was estimated by a single-factor analysis of variance with R general linear model (GLM) by setting marker loci as independent variable and each phenotypic trait as dependent variable. The R^2 values from the resulting GLM were accepted as the percentage of phenotypic variation explained by the identified QTL.

4.0 RESULTS

4.1 Weather conditions in 2010 and 2011

Average temperature and total precipitation during the growing season (May to August) in 2010 and 2011 for Saskatoon and Rosthern are given in Table 4.1. Saskatoon received more precipitation in 2010 but less in 2011 than Rosthern, and the 2010 growing season was wetter than the 2011 growing season at both locations.

Table 4.1. Summary of growing season (May-August) mean temperature and total precipitation at Saskatoon and Rosthern, Saskatchewan in 2010 and 2011

Location	Soil zone	Mean temperature (℃)	Total precipitation (mm)
2010 Saskatoon [a]	Dark Brown	16.9	387
2010 Rosthern	Black	14.9 [b]	321 [c]
2011 Saskatoon [a]	Dark Brown	15.4	195
2011 Rosthern	Black	15.7 [b]	248 [c]

[a] based on data from Environment Canada, [b] based on data from The Weather Network, [C] based on data from Saskatchewan Ministry of Agriculture weekly crop reports

4.2 Phenotyping results

In comparing the four ratings of mycosphaerella blight and the five ratings of lodging at each location-year, the third rating of mycosphaerella bight (MB3) (scored when the first RILs reached physiological maturity) and the fifth rating of lodging (LG5) (scored on the date of physiological maturity for individual RILs) had lower CVs and/or larger variation among RILs and parents than the other ratings of mycosphaerella blight and lodging, respectively (Appendix 1 and Appendix 2). Often the fourth rating of mycosphaerella blight (MB4) reached the top of the 1-9 scale, thus small differences which had been apparent at MB3 were diminished in MB4. Therefore, MB3 and LG5 were utilized in phenotypic data analysis and in QTL analysis for mycosphaerella blight resistance and lodging resistance, respectively.

Variance for seed dimpling (SD) was heterogeneous between 2010 and 2011, thus, analysis of variance for this trait were analyzed separately by year. Variance for Se concentration was heterogeneous between the two locations (Saskatoon and Rosthern)

and years (2010 and 2011), so data for Se concentration were analyzed separately for each location-year.

4.2.1 Mycosphaerella blight, AUDPC, lodging and AULPC

4.2.1.1 Mycosphaerella blight, AUDPC, lodging and AULPC in 2010

Genotypes differed significantly for MB3, AUDPC, LG5 and AULPC (Table 4.2). Location significantly affected LG5 and AULPC, but not MB3 and AUDPC. The genotype-by-location interaction was significant for MB3, but not for AUDPC, LG5 and AULPC. CVs for these three traits ranged from 6% to 14%.

Table 4.2. Analysis of variance with F values and significance levels for MB3, AUDPC, LG5 and AULPC in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010.

		F values			
Effect	numDF	MB3	AUDPC	LG5	AULPC
Genotype	143	3.68***	3.66***	2.21**	3.22***
Location	1	0.34NS	0.64NS	114.62***	107.23***
Genotype x Location	143	1.31*	1.23NS	1.02NS	1.09NS
CV (%)		8	6	12	14

Notes: MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging; NS, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$.

4.2.1.2 Mycosphaerella blight, AUDPC, lodging and AULPC in 2011

Genotypes differed significantly for MB3, AUDPC, LG5 and AULPC (Table 4.3). Location significantly affected MB3, AUDPC, LG5 and AULPC as well. The genotype-by-location interaction was significant for MB3, AUDPC and LG5, but not for AULPC. CVs for these three traits ranged from 6% to 19%.

Table 4.3. Analysis of variance with F values and significance levels for MB3, AUDPC, LG5 and AULPC in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2011.

		F values			
Effect	numDF	MB3	AUDPC	LG5	AULPC
Genotype	143	1.81***	1.89***	2.12***	4.17***
Location	1	353.87***	362.97***	455.64***	350***
Genotype x Location	143	1.46**	2.07***	1.61***	1.19NS
CV (%)		6	6	14	19

Notes: MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging; NS, not significant; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$.

4.2.1.3 Mycosphaerella blight , AUDPC, lodging and AULPC in 2010 and 2011

Genotypes differed significantly for MB3, AUDPC, LG5 and AULPC (Table 4.4). Year and location also had significant effects on these traits. The genotype-by-year interaction, the genotype-by-location interaction and the genotype-by-year-by-location interaction were significant for these three traits as well, except the genotype-by-location interaction which was not significant for LG5 and AULPC. CVs for these traits ranged from 4% to 15%.

Table 4.4. Analysis of variance with F values and significance levels for MB3, AUDPC, LG5 and AULPC in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010 and 2011.

		F values			
Effect	numDF	MB3	AUDPC	LG5	AULPC
Genotype	143	3.82***	3.91***	2.38***	5.82***
Year	1	16.60***	545.80***	45.57***	251.92***
Location	1	196.28***	273.86***	69.34***	504.20***
Genotype x Year	143	1.55***	1.44**	1.83***	1.91***
Genotype x Location	143	1.33*	1.38**	1.45NS	0.91NS
Genotype x Year x Location	143	1.38**	1.80***	1.54***	1.44**
CV (%)		6	4	10	15

Notes: MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging; NS, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$.

4.2.2 Selenium concentration

4.2.2.1 Selenium concentration in 2010

Genotype had a significant effect on Se concentration at both Saskatoon and Rosthern in 2010 (Table 4.5). The CVs were 29% and 27% at Saskatoon and Rosthern, respectively.

Table 4.5. Analysis of variance with F values and significance levels for selenium (Se) concentration in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010.

		F values	
Effect	numDF	Se_Saskatoon	Se_Rosthern
Genotype	143	2.01***	1.47*
CV (%)		29	27

Notes: *, significant at $p \leq 0.05$; ***, significant at $p \leq 0.001$.

4.2.2.2 Selenium concentration in 2011

Genotype had a significant effect on Se concentration at Saskatoon but not at Rosthern in 2011 (Table 4.6). The CVs were 39% and 41% at Saskatoon and Rosthern, respectively.

Table 4.6. Analysis of variance with F values and significance levels for selenium (Se) concentration in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2011.

		F values	
Effect	numDF	Se_Saskatoon	Se_Rosthern
Genotype	143	1.58**	1.03NS
CV (%)		39	41

Notes: 1. NS, not significant; **, significant at p≤0.01; ***.

4.2.3 Zinc and Iron concentration

4.2.3.1 Zinc and Iron concentration in 2010

Genotypes differed significantly for Zn and Fe concentration in 2010 (Table 4.7). Zn and Fe concentration were significantly affected by location and the genotype-by-location interaction was significant in all cases. The CVs for Zn and Fe concentration were 10% and 7%, respectively. The variation in Zn and Fe concentration of the two parents and among RILs in 2010 was summarized (Appendix 3).

Table 4.7. Analysis of variance with F values and significance levels for zinc (Zn) and iron (Fe) concentration in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010.

		F values	
Effect	numDF	Zn	Fe
Genotype	143	10.06***	3.69***
Location	1	644.08***	618.03***
Genotype X Location	143	1.44**	1.28*
CV (%)		10	7

Notes: *, significant at p≤0.05; **, significant at p≤0.01; ***, significant at p≤0.001.

4.1.3.2 Zinc and Iron concentration in 2011

Genotypes differed significantly for Zn and Fe concentration in 2011 (Table 4.8). Zn and Fe concentration were significantly affected by location, while the genotype-by-location interaction was not significant. The CVs for Zn and Fe concentration were 8% and 6%, respectively. The variation in Zn and Fe concentration of the two parents and among RILs in 2011 was summarized (Appendix 3).

Table 4.8. Analysis of variance with F values and significance levels for zinc (Zn) and iron (Fe) concentration in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2011.

		F values	
Effect	numDF	Zn	Fe
Genotype	143	1,72***	2.30***
Location	1	189.18***	154.76***
Genotype x Location	143	0.71NS	0.86NS
CV (%)		8	6

Notes: NS, not significant; ***, significant at $p \leq 0.001$)

4.1.3.3 Zinc and iron concentration in 2010 and 2011

Genotypes differed significantly for Zn and Fe concentration (Table 4.9). Year and location significantly affected the concentration of these two micronutrients, except for location on Zn concentration. The genotype-by-year, the genotype-by-location and the genotype-by-year-by-location interactions were not significant for Zn and Fe concentration (Table 4.9). The CVs for Zn and Fe concentration were 8% and 7%, respectively. The variation in Zn and Fe concentration of the two parents and among RILs in 2010 and 2011 was summarized (Appendix 3).

Table 4.9. Analysis of variance with F values and significance levels for zinc (Zn) and iron (Fe) concentration in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010 and 2011.

		F values	
Effect	numDF	Zn	Fe
Genotype	143	5.84***	4.77***
Year	1	761.07***	181.41***
Location	1	3.25NS	83.75***
Genotype X Year	143	1.21NS	0.99NS
Genotype X Location	143	0.75NS	0.86NS
Genotype X Year X Location	143	0.98NS	1.21NS
CV (%)		8	7

Notes: NS, not significant; ***, significant at $p \leq 0.001$)

4.2.4 Other agronomic traits

4.2.4.1 Other agronomic traits in 2010

Genotypes differed significantly for all of the agronomic traits in 2010 listed in Table 4.10. All traits except DM and PS were significantly affected by location. The genotype-by-location interaction was significant for all traits except DF, SS and SD. CVs for these traits ranged from 3% for DM to 93% for SD. The variation between the parents and among the RILs for these traits in 2010 was summarized (Appendix 4).

Table 4.10. Analysis of variance with F values and significance levels for days to flowering (DF), days to maturity (DM), plant stand (PS), light penetration (LP), plant height (HT), seed yield (Yld), 1000 seed weight (TSW), seed shape (SS) and seed dimpling (SD) in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010.

		F values								
Effect	numDF	DF	DM	PS	LP	HT	Yld	TSW	SS	SD
Genotype	143	2.91***	3.10***	2.65***	2.05***	5.89***	16.98***	30.77***	4.36***	3.50***
Location	1	10.76**	0.90NS	2.65NS	249.24***	12.49***	30.17**	3400.36***	4.60*	17.64**
Genotype x Location	143	1.21NS	1.40**	1.27*	1.45**	1.38*	1.84***	2.94***	1.17NS	0.95NS
CV (%)		6	3	17	44	17	25	11	15	93

Notes: NS, not significant; *, significant at p≤0.05; **, significant at p≤0.01; ***, significant at p≤0.001.

4.2.4.2 Other agronomic traits in 2011

Genotypes differed significantly for all of the agronomic traits in 2011 listed in Table 4.11. All traits except DF, PS, LP and SD were significantly affected by location. The genotype-by-location interaction was significant for DF, DM, Yld, TSW and SD. CVs for these traits ranged from 2% for DF to 95% for SD. The variation between the parents and among the RILs for these traits in 2011 was summarized (Appendix 4).

Table 4.11. Analysis of variance with F values and significance levels for days to flowering (DF), days to maturity (DM), plant stand (PS), light penetration (LP), plant height (HT), seed yield (Yld), 1000 seed weight (TSW), seed shape (SS) and seed dimpling (SD) in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2011.

		F values								
Effect	numDF	DF	DM	PS	LP	HT	Yld	TSW	SS	SD
Genotype	143	1.60***	2.29***	4.23***	1.69***	5.49***	7.74***	8.26***	9.06***	10.87***
Location	1	11.80NS	10.13*	0.91NS	0.03NS	147.12***	498.40**	132.70***	12.18**	1.08NS
Genotype x Location	143	2.9***	1.31*	1.23NS	1.23NS	0.74NS	1.67***	2.30***	1.21NS	2.46***
CV (%)		2	3	17	35	17	25	10	15	95

Notes: NS, not significant; *, significant at p≤0.05; **, significant at p≤0.01; ***, significant at p≤0.001.

4.2.4.3 Other agronomic traits in 2010 and 2011

Genotypes differed significantly for all agronomic traits listed in Table 4.12. Year significantly affected all traits. Location only had a significant effect on DM, LP, HT, Yld, and TSW. The genotype-by-year interaction was significant for all traits. The genotype-by-location interaction was significant for Yld, and TSW. The genotype-by-year-by-location interaction was significant for all traits except HT (Table 4.12). CVs for these traits ranged from 3% for days to maturity to 56% for light penetration. The variation between the parents and among the RILs for these traits in 2010 and 2011 was summarized (Appendix 4).

Table 4.12. Analysis of variance with F values and significance levels for days to flowering (DF), days to maturity (DM), plant stand (PS), light penetration (LP), plant height (HT), seed yield (Yld), 1000 seed weight (TSW) and seed shape (SS) in the pea recombinant inbred line population derived from Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010 and 2011.

		F values							
Effect	numDF	DF	DM	PS	LP	HT	Yld	TSW	SS
Genotype	143	3.40***	3.85***	4.33***	2.49***	9.38***	19.02***	23.83***	10.41***
Year	1	389.50***	154.12***	20.06***	6.71**	13.16*	744.12***	8.43*	233.76***
Location	1	2.90NS	8.64**	3.26NS	129.53***	57.44***	979.10***	1310.91***	0.08NS
Genotype x Year	143	2.10***	1.45**	2.01***	1.27*	1.60***	2.16***	2.27***	1.49***
Genotype x Location	143	1.20NS	2.66NS	1.04NS	1.15NS	1.04NS	1.30*	1.51***	1.07NS
Genotype x Year x Location	143	1.80***	1.57***	1.33*	1.48***	0.82NS	2.01***	3.31***	1.29*
CV (%)		5	3	17	38	20	22	10	16

Note: NS, not significant; *, significant at p≤0.05; **, significant at p≤0.01; ***, significant at p≤0.001.

4.2.5 Frequency distributions

The moderately resistant parent (CDC Striker) had mean mycosphaerella blight (MB3) score 1.5 and 1.3 units lower than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference (LSD) $_{0.05}$ = 0.9 at both locations]. Thus, differences between the parents were significant at both locations. The mycosphaerella blight scores for the RILs varied from 5.5 to 8.0 at Saskatoon and 5.5 to 8.5 at Rosthern (Fig. 4.1).

The mean area under the disease progress curve for CDC Striker and Carrera differed significantly by 35.9 [Least Significant Difference (LSD) $_{0.05}$ = 16.7] and 42.0 [Least Significant Difference (LSD) $_{0.05}$ = 18.4] at Saskatoon and Rosthern, respectively, in 2010 and 2011. Thus, differences between the parents were significant at both locations. AUDPC among the RILs varied from 138.3 to 192.5 at Saskatoon and from 160.1 to 205.6 at Rosthern (Fig. 4.1).

CDC Striker and Carrera differed by 2.5 and 2.3 units in lodging (LG5) score at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference (LSD) $_{0.05}$ = 1.9 and 1.7, respectively]. Thus, differences between the parents were significant at both locations. The variation in RILs ranged from 3.8 to 8.3 at Saskatoon and from 4.5 to 8.5 at Rosthern (Fig. 4.2).

Area under the lodging progress curve was 25.4 and 27.1 units lower in CDC Striker than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference (LSD) $_{0.05}$ = 24.7 and 38.6, respectively]. Thus, differences between the parents were significant at Saskatoon but not at Rosthern. The variation in RILs ranged from 65.6 to 149.6 at Saskatoon and from 91.0 to 190.8 at Rosthern (Fig. 4.2).

Selenium concentration was 0.19 ppm [Least Significant Difference (LSD) $_{0.05}$ = 1.31] lower in CDC Striker (1.84 ppm) than in Carrera (2.03ppm) at Saskatoon, but 0.12 ppm [Least Significant Difference (LSD) $_{0.05}$ = 0.32] higher in CDC Striker (0.47 ppm) than Carrera (0.35 ppm) at Rosthern in 2010 and 2011. Thus, differences between the parents were not significant at either location. The variation in RILs ranged from 1.16 to 4.35 ppm at Saskatoon and 0.18 to 0.81 ppm at Rosthern (Fig. 4.3).

Mean zinc concentration was higher in Carrera than CDC Striker by 4.30 ppm [Least Significant Difference (LSD) $_{0.05}$ = 4.45] and 5.05 ppm [Least Significant Difference (LSD) $_{0.05}$ = 2.76] at Saskatoon and Rosthern, respectively, in 2010 and 2011. Thus, differences between the parents were significant at Rosthern but not at Saskatoon. The variation in RILs ranged from 25.45 to 37.71 ppm at Saskatoon and from 25.08 to 38.15 ppm at Rosthern (Fig. 4.4).

Mean iron concentration was higher in Carrera than CDC Striker by 5.99 ppm [Least Significant Difference (LSD) $_{0.05}$ = 5.26] and 7.60 ppm [Least Significant Difference (LSD) $_{0.05}$ = 5.24] at Saskatoon and Rosthern, respectively, in 2010 and 2011. Thus, differences between the parents were significant at both locations. The variation in RILs varied from 41.85 to 58.80 at Saskatoon and from 39.13 to 58.80 and was skewed toward higher Fe concentration at both locations (Fig. 4.4).

CDC Striker was 0.2 and 5.7 days later to flower than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference (LSD) $_{0.05}$ = 2.6 and 3.3, respectively]. Thus, differences between the parents were significant at Rosthern but not at Saskatoon. The variation in RILs ranged from 51.8 to 58.0 days at both Saskatoon and Rosthern (Fig. 4.5).

CDC Striker was 2.5 and 0.5 days earlier to maturity than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference (LSD) $_{0.05}$ = 2.7

and 3.5, respectively]. Thus, differences between the parents were not significant at either location. The variation in RILs ranged from 93.5 to 100.5 at Saskatoon and from 93.0 to 103.0 at Rosthern (Fig. 4.5).

CDC Striker was 0.5 units lower in plant stand than Carrera at Saskatoon and 0.5 units higher than Carrera at Rosthern in 2010 and 2011 [Least Significant Difference $(LSD)_{0.05}$ = 1.33 and 1.42, respectively]. Thus, differences between the parents were not significant at either location. The variation in RILs ranged from 4.8 to 8.5 at Saskatoon and from 4.0 to 8.8 at Rosthern (Fig. 4.6).

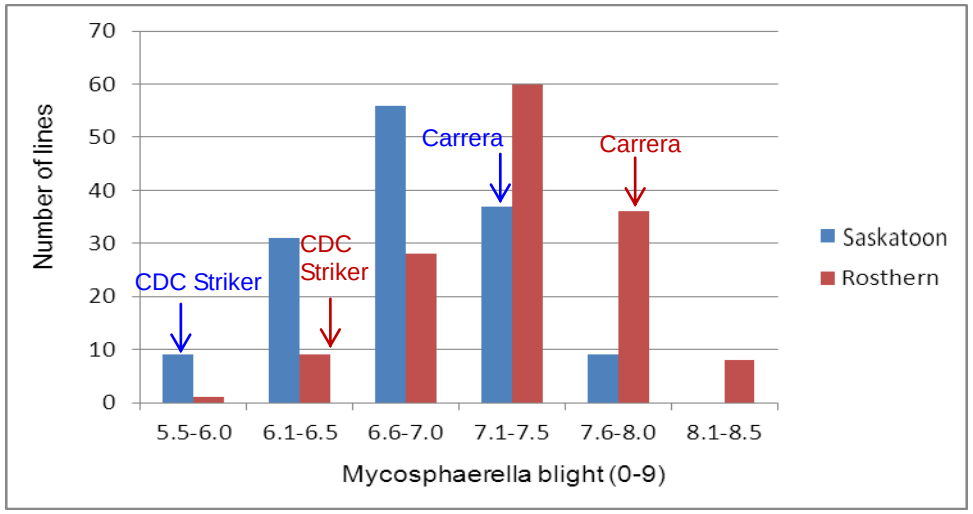
CDC Striker was 11.0 and 19.1 cm taller in plant height than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference $(LSD)_{0.05}$ = 11.9 at both locations]. Thus, differences between the parents were significant at Rosthern but not at Saskatoon. The variation in RILs ranged from 45.5 to 82.9 cm at Saskatoon and from 47.5 to 91.2 cm at Rosthern and was skewed toward greater plant height at both locations (Fig. 4.7).

CDC Striker was 33.4 g m^{-2} lower in yield than Carrera at Saskatoon and 55.4 g m^{-2} greater than Carrera at Rosthern in 2010 and 2011 [Least Significant Difference $(LSD)_{0.05}$ = 102.8 and 112.4, respectively]. Thus, differences between the parents were not significant at either location. The variation in RILs ranged from 133.0 to 555.9 at Saskatoon and from 213.9 to 694.1 at Rosthern (Fig. 4.8).

CDC Striker had mean thousand seed weight 40.3 and 36.9 grams lower than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference $(LSD)_{0.05}$ = 23.4 and 15.5, respectively]. Thus, differences between the parents were significant at both locations. The variation for the RILs varied from 201.9 to 298.5 at Saskatoon and 224.4 to 324.5 at Rosthern (Fig. 4.9).

CDC Striker was 0.5 and 0.8 units lower in seed shape than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference (LSD) $_{0.05}$ = 0.5 and 0.3, respectively]. Thus, differences between the parents were significant at both locations. The variation in RILs ranged from 2.4 to 3.8 at Saskatoon and from 2.0 to 3.9 at Rosthern (Fig. 4.10).

CDC Striker was 10% and 12% lower in seed dimpling than Carrera at Saskatoon and Rosthern, respectively, in 2010 and 2011 [Least Significant Difference (LSD) $_{0.05}$ = 8.3 and 7.1, respectively]. Thus, differences between the parents were significant at both locations. The variation in RILs ranged from 1.3 to 30.0 at Saskatoon and from 0 to 42.5 at Rosthern and was skewed toward greater seed dimpling at both locations (Fig. 4.10).



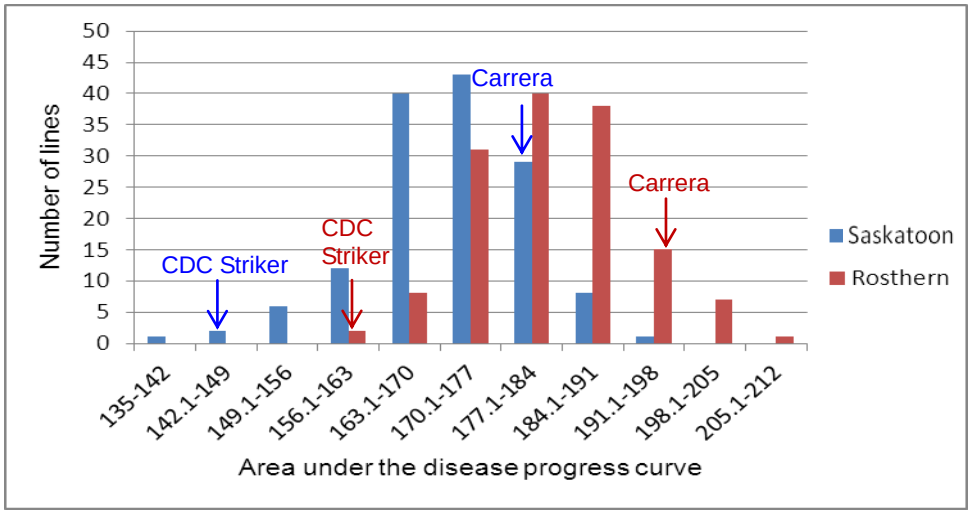
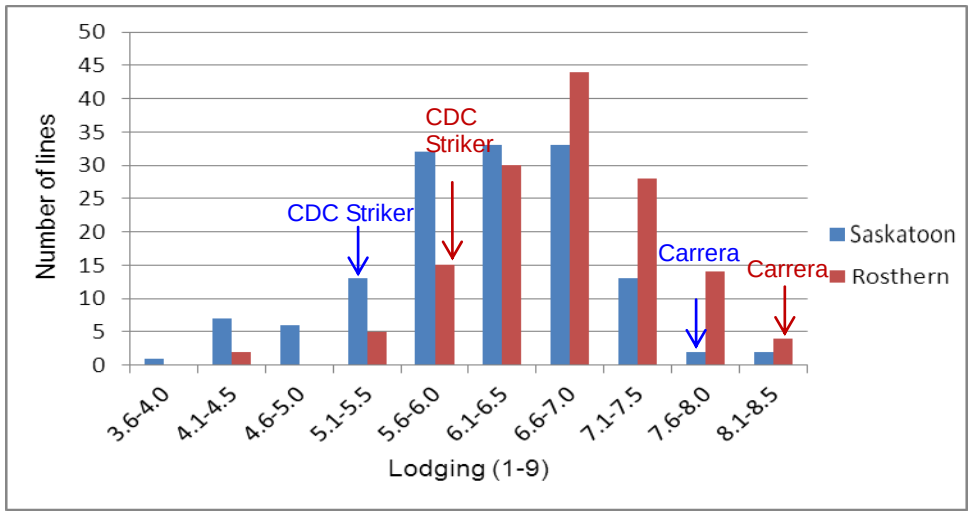


Fig 4.1. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their third rating of mycosphaerella blight (left) and area under the disease progress curve of mycosphaerella blight (right) based on the means of 2010 and 2011. **Mycosphaerella blight (left)** - Saskatoon: Mean RILs = 6.9; $LSD_{0.05}$ = 0.9; Rosthern: Mean RILs = 7.4; $LSD_{0.05}$ = 0.9. **Area under the disease progress curve (right)** - Saskatoon: Mean RILs = 171.5; $LSD_{0.05}$ = 16.7; Rosthern: Mean RILs = 182.7; $LSD_{0.05}$ = 18.4.



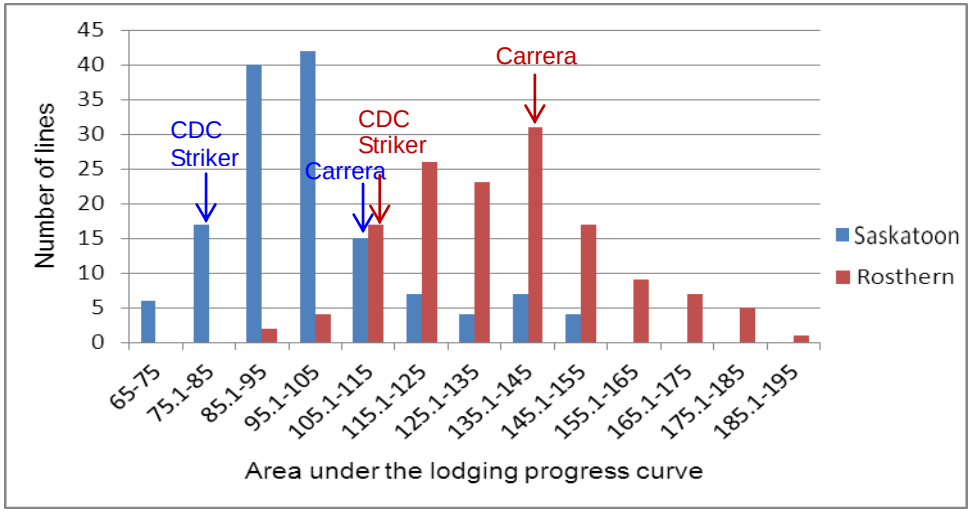


Fig. 4.2. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their fifth rating of lodging (left) and area under the lodging progress curve (right) based on the means of 2010 and 2011. **Lodging (left)** - Saskatoon: Mean RILs = 6.2; $LSD_{0.05}$ = 1.9; Rosthern: Mean RILs = 6.8; $LSD_{0.05}$ = 1.7. **Area under the lodging progress curve (right)** - Saskatoon: Mean RILs = 99.7; $LSD_{0.05}$ = 24.7; Rosthern: Mean RILs = 133.6; $LSD_{0.05}$ = 38.6.

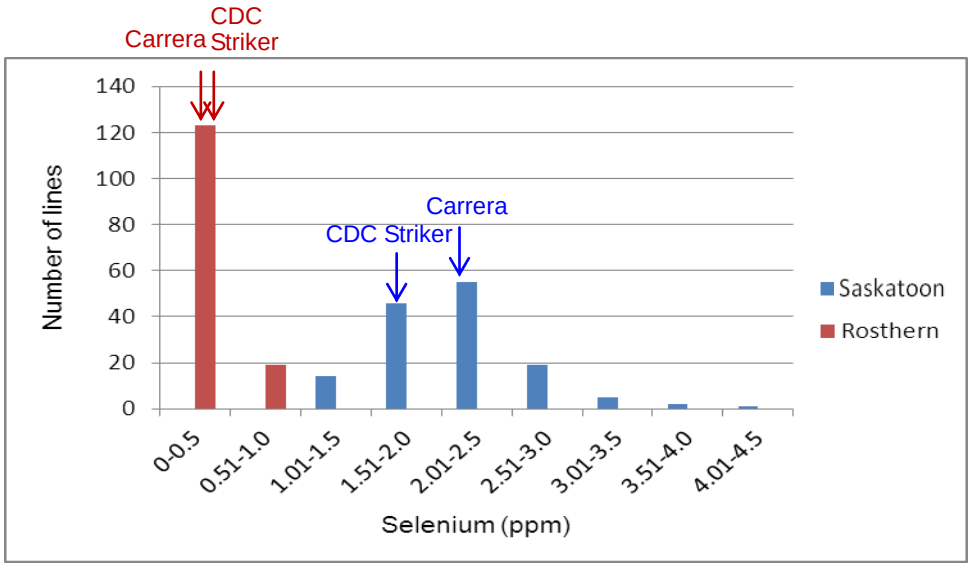
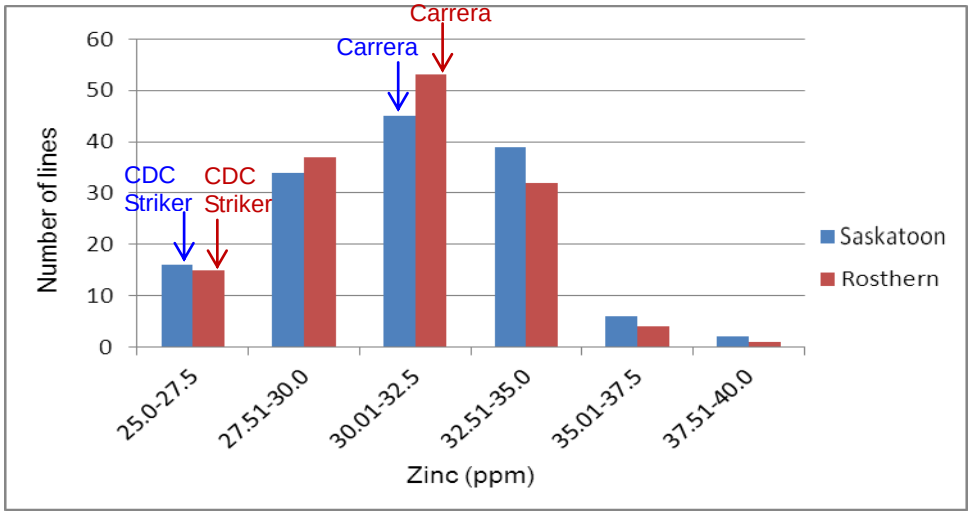


Fig. 4. 3. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their selenium concentration based on the means of 2010 and 2011. Saskatoon: Mean RILs = 2.14; $LSD_{0.05}$ = 1.31; Rosthern: Mean RILs = 0.40; $LSD_{0.05}$ = 0.32.



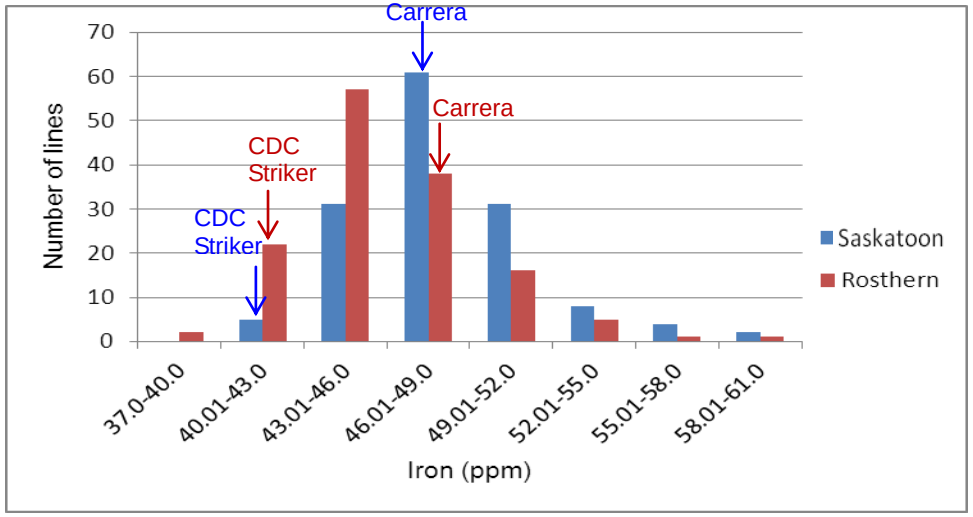
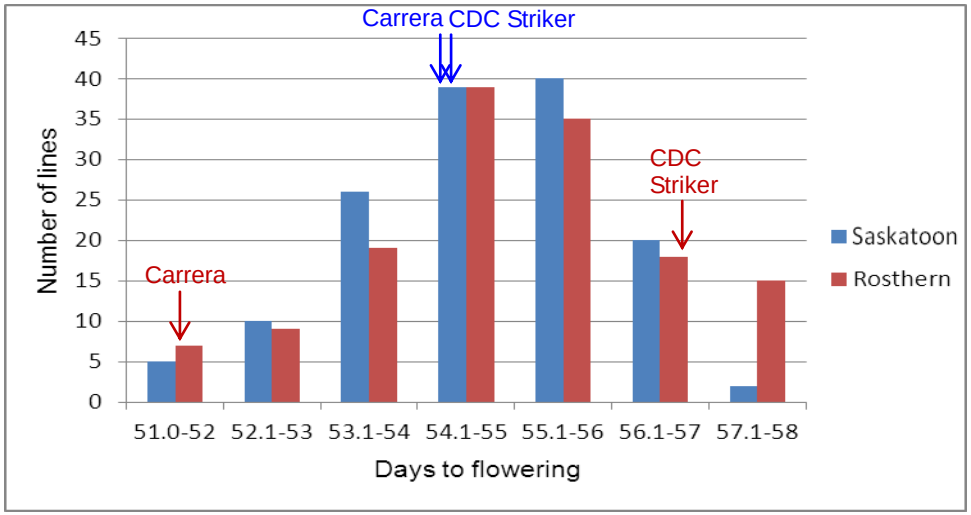


Fig. 4.4. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their zinc concentration (left) and iron concentration (right) based on the means of 2010 and 2011. **Zinc concentration (left)** - Saskatoon: Mean RILs = 31.1; $LSD_{0.05}$ = 4.45; Rosthern: Mean RILs = 30.8; $LSD_{0.05}$ = 2.76. **Iron concentration (right)** - Saskatoon: Mean RILs = 47.9; $LSD_{0.05}$ = 5.26; Rosthern: Mean RILs = 45.8; $LSD_{0.05}$ = 5.24.



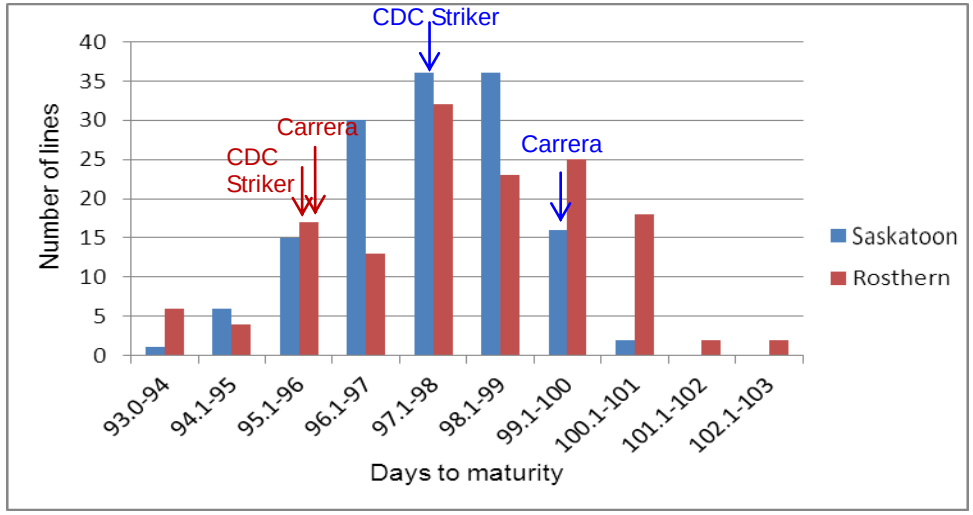
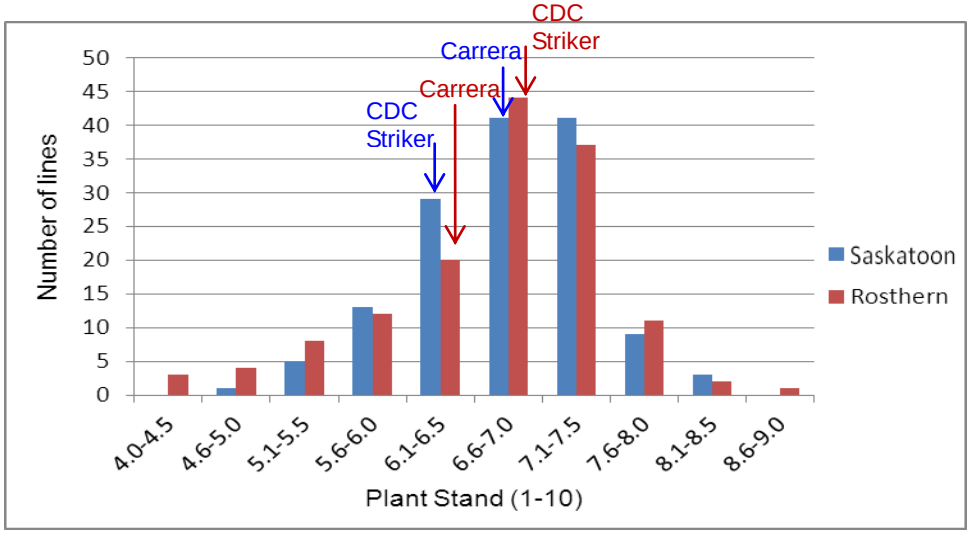


Fig. 4.5. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their days to flowering (left) and days to maturity (right) based on the means of 2010 and 2011. **Days to flowering (left)** - Saskatoon: Mean RILs = 54.8; $LSD_{0.05}$ = 2.6; Rosthern: Mean RILs = 55.0; $LSD_{0.05}$ = 3.3. **Days to maturity (right)** - Saskatoon: Mean RILs = 97.7; $LSD_{0.05}$ = 2.7; Rosthern: Mean RILs = 98.1; $LSD_{0.05}$ = 3.5.



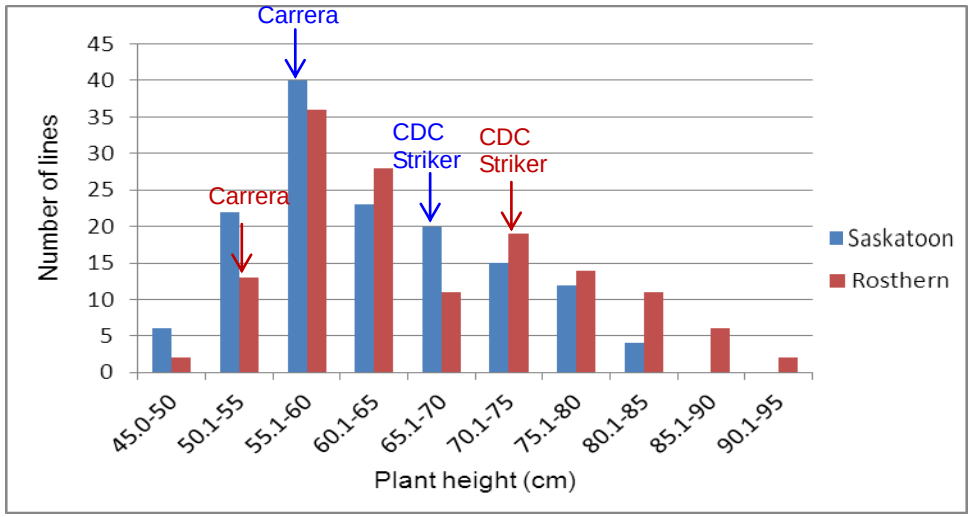


Fig. 4.6. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their plant stand based on the means of 2010 and 2011. Saskatoon: Mean RILs = 6.8; $LSD_{0.05}$ = 1.3; Rosthern: Mean RILs = 6.8; $LSD_{0.05}$ = 1.4.

Fig. 4.7. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their plant height based on the means of 2010 and 2011. Saskatoon: Mean RILs = 62.6; $LSD_{0.05}$ = 11.9; Rosthern: Mean RILs = 66.5; $LSD_{0.05}$ = 11.9.

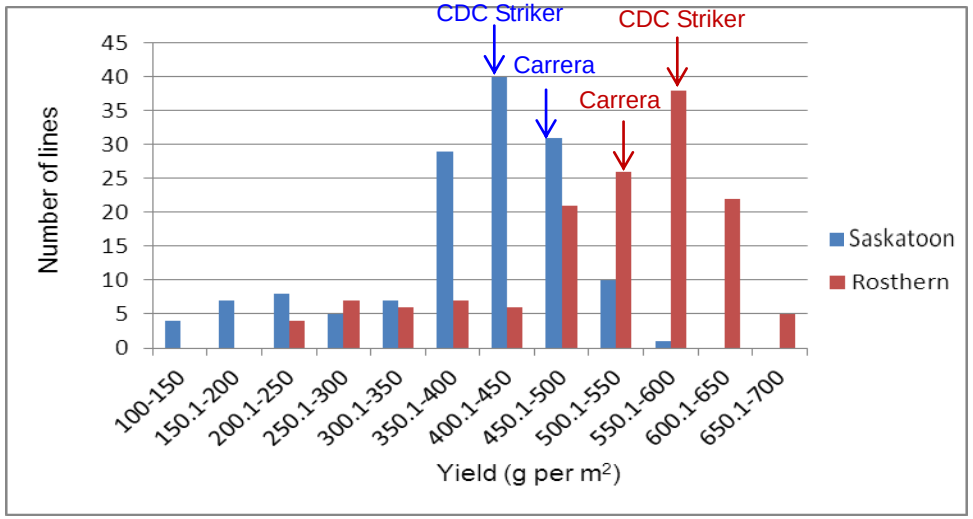


Fig. 4.8. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their yield based on the means of 2010 and 2011. Saskatoon: Mean RILs = 390.9; $LSD_{0.05}$ = 102.8; Rosthern: Mean RILs = 510.4; $LSD_{0.05}$ = 112.4.

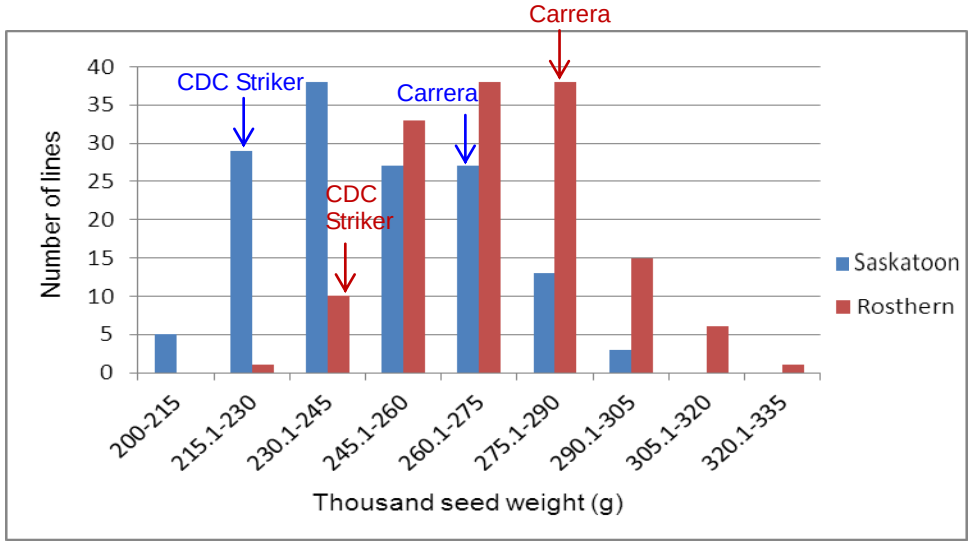
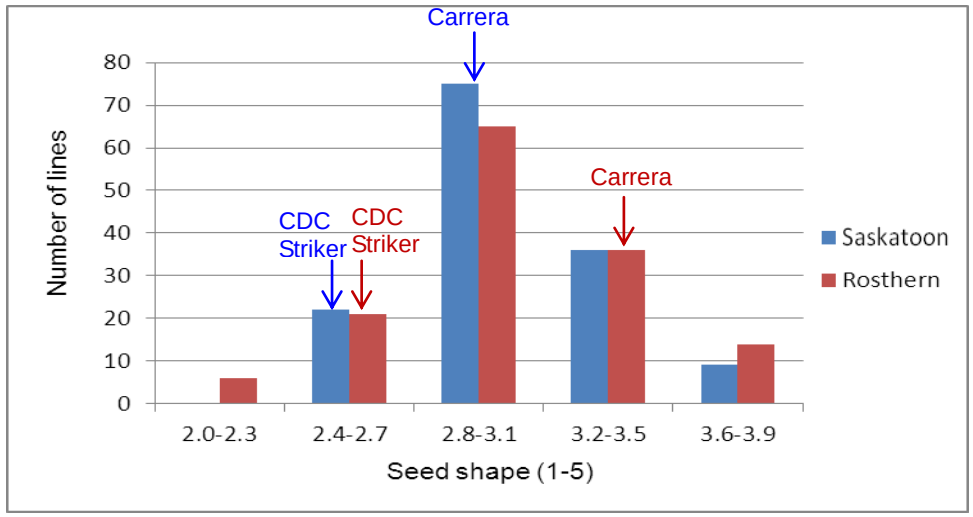


Fig. 4.9. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their thousand seed weight based on the means of 2010 and 2011. Saskatoon: Mean RILs = 247.1; $LSD_{0.05}$ = 23.4; Rosthern: Mean RILs = 271.2; $LSD_{0.05}$ = 15.5.



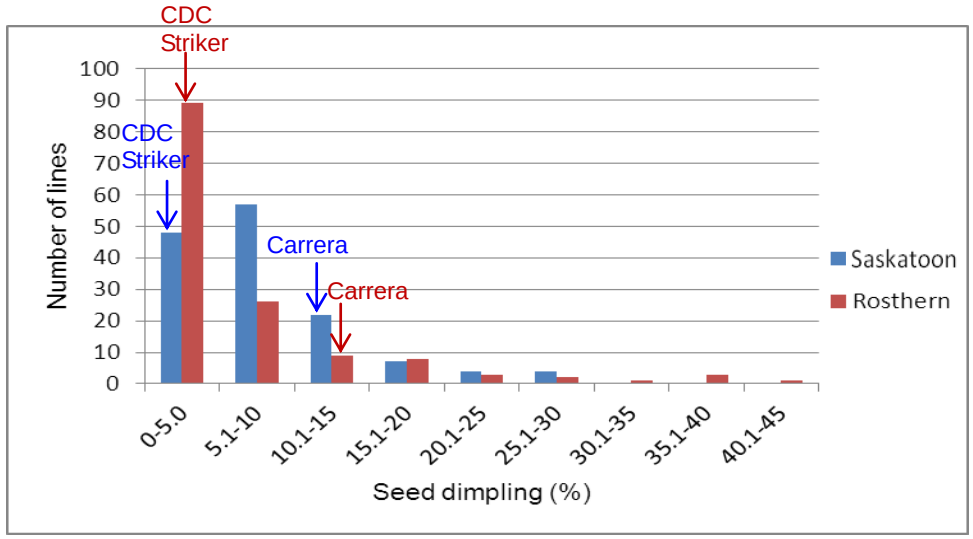


Fig. 4.10. Frequency distribution of 142 recombinant inbred lines (RILs) derived from Carrera/CDC Striker for their seed shape (left) and percentage of seed dimpling (right) based on the means of 2010 and 2011. **Seed shape (left)** - Saskatoon: Mean RILs = 3.0; $LSD_{0.05}$ = 0.5; Rosthern: Mean RILs = 3.0; $LSD_{0.05}$ = 0.3. **Seed dimpling (right)** - Saskatoon: Mean RILs = 8.3; $LSD_{0.05}$ = 11.1; Rosthern: Mean RILs = 7.0; $LSD_{0.05}$ = 7.1.

4.2.6 Correlations

4.2.6.1 Correlations in 2010

Table 4.13 shows the correlation coefficients among the agronomic traits. Significant (r= 0.75, P<0.001) correlation was observed between MB3 and AUDPC. Mycosphaerella blight (MB3) was positively correlated with lodging (LG5) (r= 0.26, P<0.001), but negatively correlated with plant height (r= -0.23, P<0.001). AUDPC was positively correlated with AULPC (r= 0.16, P<0.001). LG5 was positively correlated with AULPC (r= 0.51, P<0.001) and negatively correlated with plant height. Light penetration through the canopy was positively correlated with MB3 (r= 0.19, P<0.001) and AUDPC (r= 0.10, P<0.001). Days to flowering was negatively correlated with MB3 (r= -0.20, P<0.001), AUDPC (r= -0.26, P<0.001) and LG5 (r= -0.11, P<0.001). Days to maturity was negatively correlated with MB3 (r= -0.38, P<0.001), AUDPC (r= -0.42, P<0.001) and LG5 (r= -0.18, P<0.001). In addition, positive correlation (r= 0.29, P<0.001) between days to flowering and days to maturity was observed.

Table 4.13. Correlation between MB3, AUDPC, LG5, AULPC, HT, LP, DF and DM for the 142 recombinant inbred lines of the Carrera/CDC Striker population evaluated at Saskatoon and Rosthern in 2010 based on raw data.

	MB3	AUDPC	LG5	AULPC	HT	LP	DF	DM
MB3	\	0.75**	0.26***	0.04NS	-0.23***	0.19***	-0.20***	-0.38***
AUDPC		\	0.16***	0.16***	-0.17***	0.10*	-0.26***	-0.42***
LG5			\	0.51***	-0.09*	-0.24***	-0.11***	-0.18***
AULPC				\	0.23***	-0.28***	-0.03NS	0.08*
HT					\	-0.30***	-0.04NS	-0.12***
LP						\	0NS	-0.02NS
DF							\	0.29***
DM								\

Notes: MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging, HT= plant height, LP= light penetration, DF= days to flowering, DM= days to maturity; NS, not significant; *, significant at p≤0.05; **, significant at p≤0.01; ***, significant at p≤0.001.

4.2.6.2 Correlations in 2011

Table 4.14 shows the correlation coefficients among the agronomic traits. Significant correlations were observed between mycosphaerella blight (MB3) and AUDPC (r= 0.80, P<0.001), MB3 and lodging (LG5) (r= 0.27, P<0.001), as well as MB3 and AULPC (r= 0.41, P<0.001). AUDPC was positively correlated with LG5 (r = 0.32, P<0.001) and AULPC (r= 0. 54, P<0.001). LG5 was positively correlated with AULPC (r= 0.77, P<0.001). In 2011, unlike 2010, a significantly positive correlation was observed between LG5 and plant height (r= 0.33, P<0.001). Light penetration was positively related with AUDPC (r= 0.15, P<0.001). Days to flowering was negatively correlated with AULPC (r= -0.18, P<0.001) and AUDPC (r= -0.27, P<0.001), indicating plants with earlier flowering tended to have greater severity of mycosphaerella blight. AUDPC was negatively correlated with days to maturity (r= -0.26, P<0.001). In addition, positive correlation between days to flowering and days to maturity (r= 0.38, P<0.001) was observed.

Table 4.14. Correlation between MB3, AUDPC, LG5, AULPC, HT, LP, DF and DM for the 142 recombinant inbred lines of the Carrera/CDC Striker population evaluated at Saskatoon and Rosthern in 2011 based on raw data.

	MB3	AUDPC	LG5	AULPC	HT	LP	DF	DM
MB3	\	0.80***	0.27***	0.41***	0.03NS	0.04NS	-0.03NS	0.00NS
AUDPC		\	0.32***	0.54***	0.15***	0.15**	-0.27***	-0.26***
LG5			\	0.77***	0.33***	-0.06NS	-0.06NS	0.06NS
AULPC				\	0.46***	-0.02NS	-0.18***	0.02NS
HT					\	-0.29***	-0.05NS	0.19***
LP						\	-0.03NS	-0.16***
DF							\	0.38***
DM								\

Notes: MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging, HT= plant height, LP= light penetration, DF= days to flowering, DM= days to maturity; NS, not significant; *, significant at p≤0.05; **, significant at p≤0.01; ***, significant at p≤0.001.

4.2.6.3 Correlations in 2010 and 2011

Table 4.15 shows the correlation coefficients among the agronomic traits. Significant correlations were observed between mycosphaerella blight (MB3) and AUDPC (r= 0.72, P<0.001), MB3 and lodging (LG5) (r= 0.35, P<0.001), MB3 and AULPC (r= 0.26, P<0.001), LG5 and AULPC (r= 0.63, P<0.001), LG5 and plant height (r=0.16, P<0.001), as well as AULPC and plant height (r= 0.40, P<0.001). Light penetration was positively correlated with MB3 (r=0.10, P<0.001) and AUDPC (r= 0.13, P<0.001). Days to flowering was negatively correlated with lodging (r= -0.12, P<0.001). Days to flowering was also negatively correlated with MB3 (r= -0.18, P<0.001) and AUDPC (r= -0.47, P<0.001), indicating plants with earlier flowering tended to have greater severity of mycosphaerella blight. Days to maturity was negatively correlated with MB3 (r= -0.17, P<0.001) and AUDPC (r= -0.42, P<0.001). Positive correlation (r=0.40, P<0.001) between days to flowering and days to maturity was observed.

Table 4.15. Correlation between MB3, AUDPC, LG5, AULPC, HT, LP, DF and DM for the 142 recombinant inbred lines of the Carrera/CDC Striker population evaluated at Saskatoon and Rosthern in 2010 and 2011 based on raw data.

	MB3	AUDPC	LG5	AULPC	HT	LP	DF	DM
MB3	\	0.72***	0.35***	0.26***	-0.09***	0.10***	-0.18***	-0.17***
AUDPC		\	0.29***	0.25***	-0.03NS	0.13***	-0.47***	-0.42***
LG5			\	0.63***	0.16***	-0.14***	-0.12***	-0.07NS
AULPC				\	0.40***	-0.11***	0.08**	0.10**
HT					\	-0.25***	0.05NS	0.17***
LP						\	-0.04NS	-0.10**
DF							\	0.40***
DM								\

Notes: MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging, HT= plant height, LP= light penetration, DF= days to flowering, DM= days to maturity; NS, not significant; *, significant at p≤0.05; **, significant at p≤0.01; ***, significant at p≤0.001.

4.2.7 Variance components and heritability

Table 4.16 shows the variance components, their standard deviations and the broad-sense heritability estimates for those phenotypic traits measured at both locations over two years. The broad-sense heritability values ranged from 0.16 (LG5) to 0.88 (TSW). AULPC had higher heritability (0.29) than LG5 (0.16). Heritability for MB3 and AUDPC were 0.55 and 0.42, respectively. Heritability for Zn and Fe were 0.62 and 0.46, respectively. The moderate values (MB3, AUDPC, Zn and Fe) and low value (LG5 and AULPC) of broad sense heritability indicate that the expression of genes for these traits was influenced by the environment.

Table 4.16. Estimates of variance components and heritability of the traits for the 142 recombinant inbred lines of the Carrera/CDC Striker evaluated at Saskatoon and Rosthern in 2010 and 2011.

Variance component	MB3	AUDPC	LG5	AULPC	Zn	Fe
σ^2_g	0.10 ±0.31	26.80 ±5.18	0.09 ±0.00	359.3 ±18.95	3.63 ±1.91	4.10 ±2.02
σ^2_y	0.02 ±0.12	189.40 ±13.70	0.11 ±0.32	1793.7 ±42.35	10.2 ±3.19	4.72 ±2.17
σ^2_l	0.14 ±0.37	60.90 ±7.80	0.16 ±0.41	1929.6 ±43.93	0.02 ±0.11	2.10 ±1.45
σ^2_{gl}	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
σ^2_{gy}	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
σ^2_{gyl}	0.15 ±0.38	78.30 ±8.80	1.15 ±1.07	2816.3 ±53.06	2.63 ±1.62	10.23 ±3.20
σ^2_e	0.35 ±0.59	137.00 ±11.70	1.47 ±1.21	1532.4 ±39.15	12.49 ±3.53	18.11 ±4.25
σ^2_p	0.18	63.50	0.56	1254.93	5.85	8.92
H^2	0.55	0.42	0.16	0.29	0.62	0.46

Notes: MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging, Zn= Zinc, Fe= Iron, σ^2_g= genotypic variance, σ^2_y= year variance, σ^2_l= location variance, σ^2_{gl}= genotype X location interaction variance, σ^2_{gy}= genotype X year interaction variance, σ^2_{gyl}= genotype X year X location interaction variance, σ^2_e= error variance, σ^2_p= phenotypic variance, H^2= broad-sense heritability.

Table 4.16 (continued)

Variance component	DF	DM	HT	SS	TSW	Yld
σ^2G	0.51±0.71	1.37±1.17	68.62±8.28	0.10±0.32	323.96±17.99	7456.40±86.35
σ^2Y	6.95±2.64	1.16±1.08	1.40±1.19	0.04±0.19	1.33±1.15	5419.10±73.62
σ^2L	0.009±0.09	0.07±0.27	7.12±2.67	0.00±0.00	289.12±17.00	7111.80±84.33
σ^2GL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
σ^2GY	0.51±0.72	0.06±0.24	3.64±1.91	0.006±0.08	0.00±0.00	0.00±0.00
σ^2GYL	0.88±0.94	0.83±0.91	13.92±3.73	0.011±0.10	118.95±10.91	8498.60±92.19
σ^2e	3.46±1.86	4.63±2.15	71.58±8.46	0.09±0.31	130.95±11.44	4735.70±68.82
σ^2P	1.42	2.19	82.87	0.12	370.07	10173.01
H^2	0.36	0.63	0.83	0.83	0.88	0.73

Notes: DF= days to flowering, DM= days to maturity, HT= plant height, SS= seed shape, TSW= thousand seed weight, and Yld= yield, σ^2_g= genotypic variance, σ^2_y= year variance, σ^2_l= location variance, σ^2_{gl}= genotype X location interaction variance, σ^2_{gy}= genotype X year interaction variance, σ^2_{gyl}= genotype X year X location interaction variance, σ^2_e= error variance, σ^2_p= phenotypic variance, H^2= broad-sense heritability.

4.3 Genotyping results

4.3.1 Molecular marker analysis and linkage map construction

Eighty-seven out of 330 (26%) SSR markers showed polymorphism between Carrera and CDC Striker. Among these markers, 64 (74%) produced clear segregating bands among the RILs. Twenty-three markers initially identified as polymorphic between the parents either had monomorphic bands or failed to amplify in the RILs and were not further used in this study. A genetic linkage map was constructed using these 64 markers. Thirteen linkage groups (LG) were generated based on 56 (88%) SSR markers, with 8 SSR markers unlinked.

Among the 13 LGs identified in this population, 12 were aligned with 6 (LG I, LG III, LG IV, LG V, LG VI, and LG VII) of the 7 LGs previously published by Loridon et al. (2005) using common SSR markers. LG I, LG IV, LG V, LG VI, and LG VII consisted of more than one independent segment owing to lack of marker coverage, and were indicated as LG I-1, LG I-2, LG IV-1, LG IV-2, LG V-1, LG V-2, LG VI-1, LG VI-2, LG VII-1, LG VII-2, and LG VII-3. One LG (A) could not be assigned to any of the 7 LGs of the pea genome owing to lack of anchor markers mapped to these LGs (Fig.

4.11).

4.3.2 Segregation distortion analysis

The goodness-of-fit of the observed segregation ratio to the expected ratio (Chi square test, P<0.05) identified 16 (25%) loci that did not segregate in accordance with the expected Mendelian inheritance ratio of 1:1 (Table 4.17). The loci with distorted segregation ratio were located on LGIII (AA278), LG IV (AA8, AA506, AA255, B17 and AA122), LGV (AA399, AA47, AD175 and AA460), and LGVII (AD135).

Table 4.17. Segregation ratios of pea simple sequence repeat (SSR) markers that deviated from the expected 1:1 Mendelian ratio and frequency of maternal alleles in the mapping population of Carrera/CDC Striker.

Marker	Linkage group	Segregation ratio		Chi-square (P<0.05)[a]	Frequency of maternal alleles (%)
		Expected	Observed		
AA278	III	72:72	84:59	4.4	59
AA8	IV-2	71:71	51:90	10.8	36
AA506	IV-2	72:72	52:92	11.1	36
AA255	IV-2	72:72	49:94	14.2	34
B17	IV-2	72:72	58:86	5.4	40
AA122	IV-2	71:71	58:83	4.4	41
AA399	V-1	72:72	53:90	9.6	37
AA47	V-1	71:71	52:90	10.2	37
AD175	V-1	71:71	51:87	9.4	37
AA460	V-1	72:72	60:84	4.0	42
AD135	VII-3	70:70	51:88	9.8	37
A9	Unlinked	71:71	110:31	44.3	78
AA61	Unlinked	66:66	53:79	5.1	40
AA504	Unlinked	72:72	87:57	6.3	60
AD160	Unlinked	72:72	60:84	4.0	42
AD180	Unlinked	71:71	59:83	4.1	42
Mean (Range)				9.8 (4.1-14.2)	44 (36-78)

[a] χ^2 (0.05, 1) = 3.84

4.3.3 General features of the map

The general features of the genetic map developed in this study are summarized in Table 4.18. The total coverage of the map was 288.3 cM and the average distance between markers was 5.1 cM. LGV-2 represented the smallest linkage group in terms of

length and number of markers mapped, having two markers covering 2.4 cM. LGIV-2 was the longest linkage group covering 70.8 cM with an average distance between markers of 7.1 cM. The largest average distance between markers was found in LG VII-2 at 7.9 cM. The densest linkage groups with least average distance between markers were LGV-2, A, and LGI-2, which had average distance between markers of less than 3.0 cM.

Table 4.18. General features of genetic map developed using simple sequence repeats (SSRs) based on 142 recombinant inbred lines of the Carrera/CDC Striker population.

Linkage groups	Size (cM)	Number of mapped markers			Average marker distance (cM)
		Mendelian segregation	Distorted segregation	Total	
I-1	22.2	4	0	4	5.6
I-2	11.4	4	0	4	2.9
III	43.6	7	1	8	5.5
IV-1	15.6	4	0	4	3.9
IV-2	70.8	5	5	10	7.1
V-1	29.2	1	3	4	7.3
V-2	2.4	2	0	2	1.2
VI-1	11.5	3	0	3	3.8
VI-2	16.6	4	0	4	4.2
VII-1	26.6	5	0	5	5.3
VII-2	23.6	3	0	3	7.9
VII-3	9.3	2	1	3	3.1
A	5.5	2	0	2	2.8
Total/Ave	288.3	46	10	56	5.1

Note: cM= centiMorgan

4.3.4 QTL analysis of phenotypic traits

Putative QTL regions associated with agronomic, morphological and physiological traits measured at both locations in 2010 and 2011 were identified and are presented in Table 4.19.

A total of 8 genomic regions were identified over 13 LGs having association with various phenotypic traits under field conditions (Fig. 4.11). The contribution of individual QTL in terms of phenotypic variation of traits (explained as % in R^2) and additive effect for each QTL are presented in Table 4.19. A positive value means the

presence of the allele from Carrera increases the value of the phenotype, while a negative value means the phenotype from Carrera decreases the phenotypic value among the lines in the population.

A QTL for seed shape on LGI-1 (Q1-1) was identified at Saskatoon in 2011. The percentage of the seed shape phenotype explained by Q1-1 was 4.0%. The molecular marker associated with Q1-1 was SSR marker locus AA179 and round seed shape allele was contributed by CDC Striker. The AA179 allele from CDC Striker contributed 0.13 to increase the roundness of seed shape.

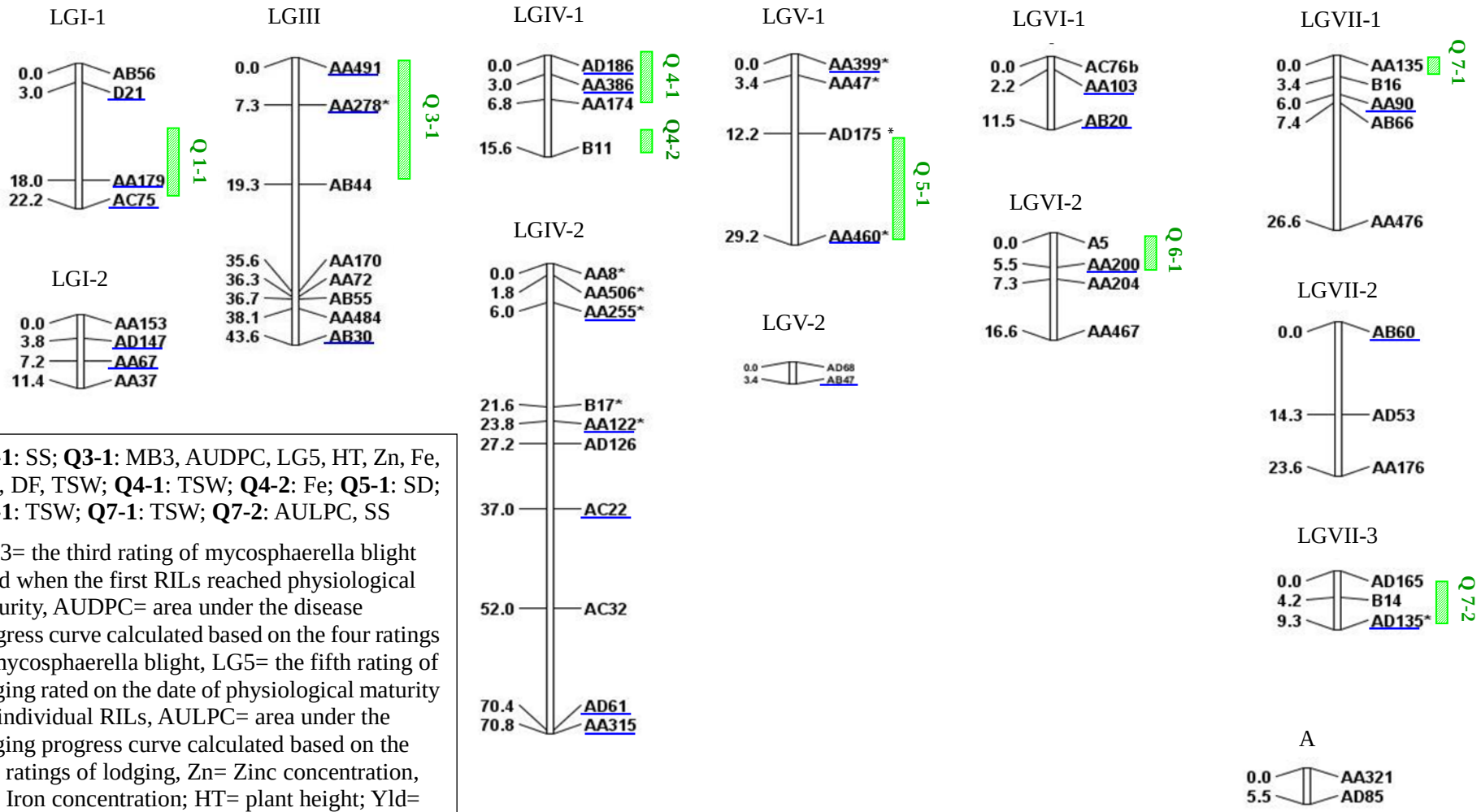
About half of LG III (Q3-1), between markers AA491 and AB44 (19.3 cM), was associated with several traits: MB3, AUDPC, LG5, zinc concentration, iron concentration, plant height, yield, days to flowering, and thousand seed weight. A QTL associated with MB3 resistance, AUDPC, LG5 resistance, zinc concentration, iron concentration and plant height was identified across both locations and both years, except for AUDPC at Saskatoon in 2010 and at Rosthern in 2011, lodging resistance at Rosthern in 2011, zinc concentration at Rosthern in 2011 and iron concentration at Saskatoon in 2011. The percentage of phenotype explained by Q3-1 ranged from 7.6% to 19.7% for MB3, 9.5% to 13.1% for AUDPC, 8.2% to 32.5% for LG5, 12.9% to 20.8% for zinc concentration, 9.6% to 16.5 % for iron concentration and 31.4% to 59.3% for plant height. Q3-1 was also associated with yield at Saskatoon in 2010 and Rosthern in 2011, days to flowering at both locations in 2011, and thousand seed weight at Saskatoon in 2011. The phenotypic variation explained by Q3-1 ranged from, 12.3% to 21.8% for yield, 15.6% to 22.5% for days to flowering, and 1.4% for thousand seed weight. The closest marker linked to this QTL was either AA491 or AA278. Additive effects of Q3-1 corresponding with specific traits at particular locations and years by their parents were listed in Table 4.19.

Q4-1 and Q4-2 on LGIV were associated with thousand seed weight at Saskatoon in 2011 and iron concentration at Saskatoon in 2011, respectively. These two QTLs explained 2.7% of the phenotypic variation for thousand seed weight and 9.4% for iron concentration. The closest markers associated with these QTLs for thousand seed weight and iron concentration were AA386 and B11, respectively. Additive effects of two QTLs were 9 and 1.1, respectively and contributed by Carrera to increase thousand seed weight and iron concentration.

Q5-1 on LGV associated with seed dimpling was detected at both locations and both years. This QTL was associated with the marker locus AA460 and explained from 15.8% to 22.9% of the phenotypic variation. Additive effects of this QTL ranged from 3.5 to 4.6, contributed from Carrera to increase the percentage of seed dimpling.

Q6-1 on LG VI-2 and Q7-1 on LGVII-1 were associated with thousand seed weight at Rosthern in both years. They explained 13.3% and 7.5% of the phenotypic variation, respectively. The allelic contributions for these two QTLs were 6.9 (AA200) and 7 (AA135) from CDC Striker and Carrera, respectively.

Q7-2 on LGVII-2 was associated with AULPC and seed shape, accounting for 13.4% to18.3% of the phenotypic variation for AULPC and 9.4% to 14.5% for seed shape. The closest marker associated with AULPC was AD 135 at both Rosthern in 2010 and Saskatoon in 2011. Additive effects of this QTL ranged from 7.7 to 12.0, contributed from Carrera to increase the value of AULPC. The closest marker loci associated with seed shape were B14 at Saskatoon in 2010, AD135 at Rosthern in 2010 and at Saskatoon in 2011, as well as AD165 at Rosthern in 2011. Alleles of this QTL were contributed by Carrera and decreased seed roundness by 0.12 to 0.18.



Q1-1: SS; **Q3-1**: MB3, AUDPC, LG5, HT, Zn, Fe, Yld, DF, TSW; **Q4-1**: TSW; **Q4-2**: Fe; **Q5-1**: SD; **Q6-1**: TSW; **Q7-1**: TSW; **Q7-2**: AULPC, SS

MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging, Zn= Zinc concentration, Fe= Iron concentration; HT= plant height; Yld= yield; DF= days to flowering; SS= seed shape; TSW= thousand seed weight; SD= seed dimpling

Fig. 4.11. Genetic linkage map of field pea (*Pisum sativum* L.) developed from 56 SSR markers based on 142 recombinant inbred line population derived from the cross between Carrera and CDC Striker. LGI to LGVII represent the linkage groups assigned to the seven previously described chromosomes of the pea genome using anchor markers indicated by underlined text. Linkage group A is unassigned owing to lack of anchor markers. The left side of the linkage groups shows the genetic distances in centiMorgans (cM) calculated based on Kosambi mapping units. Markers with asterisk (*) are deviated from 1:1 Mendelian segregation ratio at P=0.05. Vertical bars indicate the location of identified QTLs.

Table 4.19. QTLs identified for phenotypic traits based on 142 recombinant inbred lines of the Carrera/CDC Striker population evaluated at Saskatoon and Rosthern in 2010 and 2011.

QTL region	Phenotype[a]	Year	Trial location	Linkage group	Maximum LOD value	Closest marker[b]	LOD[c]	$R^{2,d}$	Add. effect[e]	Direction[f]
Q1-1	SS	2011	Saskatoon	I-1	5.1	AA179	3.4	4.0%	0.13	(Carrera)
Q3-1	MB3	2010	Saskatoon	III	5.5	AA491	3.3	16%	0.30	(Carrera)
Q3-1	MB3	2010	Rosthern	III	7.6	AA278	3.3	19.7%	0.37	(Carrera)
Q3-1	MB3	2011	Saskatoon	III	5.6	AA491	3.2	14.2%	0.27	(Carrera)
Q3-1	MB3	2011	Rosthern	III	3.5	AA278	3.3	7.6%	0.22	(Carrera)
Q3-1	AUDPC	2010	Rosthern	III	3.4	AA278	2.9	9.5%	4.30	(Carrera)
Q3-1	AUDPC	2011	Saskatoon	III	4.9	AA278	2.8	13.1%	4.91	(Carrera)
Q3-1	LG5	2010	Saskatoon	III	15	AA491	3.2	32.5%	0.61	(Carrera)
Q3-1	LG5	2010	Rosthern	III	5.2	AA278	3.3	9.2%	0.54	(Carrera)
Q3-1	LG5	2011	Rosthern	III	3.0	AA491	2.4	8.2%	0.24	(Carrera)
Q3-1	Zn	2010	Saskatoon	III	5.8	AA491	3.2	17.1%	1.70	(Carrera)
Q3-1	Zn	2010	Rosthern	III	7.2	AA491	3.2	20.8%	1.50	(Carrera)
Q3-1	Zn	2011	Saskatoon	III	4.3	AA491	3.1	12.9%	1.10	(Carrera)
Q3-1	Fe	2010	Saskatoon	III	3.2	AA278	2.6	9.6%	-1.83	(CDC Striker)
Q3-1	Fe	2010	Rosthern	III	5.6	AA278	3.2	14.7%	-1.78	(CDC Striker)
Q3-1	Fe	2011	Rosthern	III	5.6	AA278	3.1	16.5%	-1.60	(CDC Striker)
Q3-1	HT	2010	Saskatoon	III	12	AA278	3.2	31.4%	-5.66	(CDC Striker)
Q3-1	HT	2010	Rosthern	III	34	AA278	4.2	59.3%	-9.28	(CDC Striker)
Q3-1	HT	2011	Saskatoon	III	24	AA278	3.2	48.5%	-9.28	(CDC Striker)
Q3-1	HT	2011	Rosthern	III	18	AA278	3.1	39.9%	-8.98	(CDC Striker)
Q3-1	Yld	2010	Saskatoon	III	4.0	AA278	3.0	12.3%	46.00	(Carrera)
Q3-1	Yld	2011	Rosthern	III	8.0	AA491	3.1	21.8%	67.00	(Carrera)
Q3-1	DF	2011	Saskatoon	III	5.7	AA278	3.0	15.6%	-0.36	(CDC Striker)
Q3-1	DF	2011	Rosthern	III	7.9	AA278	3.0	22.5%	0.75	(Carrera)
Q3-1	TSW	2011	Saskatoon	III	4.1	AA278	3.3	1.4%	9.00	(Carrera)
Q4-1	TSW	2011	Saskatoon	IV-1	4.1	AA386	3.3	2.7%	9.00	(Carrera)

Table 4.19. Continued

QTL region	Phenotype[a]	Year	Trial location	Linkage group	Maximum LOD value	Closest marker[b]	LOD[c]	$R^{2,d}$	Add. effect[e]	Direction[f]
Q4-2	Fe	2011	Saskatoon	IV-1	3.1	B11	2.4	9.4%	1.10	(Carrera)
Q5-1	SD	2010	Saskatoon	V-1	5.3	AA460	4.5	15.8%	3.90	(Carrera)
Q5-1	SD	2010	Rosthern	V-1	7.7	AA460	7.2	22.9%	4.60	(Carrera)
Q5-1	SD	2011	Saskatoon	V-1	7.6	AA460	3.8	20.7%	3.50	(Carrera)
Q5-1	SD	2011	Rosthern	V-1	7.1	AA460	3.3	20.7%	4.60	(Carrera)
Q6-1	TSW	2010	Saskatoon	VI-2	3.5	AA200	3.3	11.7%	-7.40	(CDC Striker)
Q6-1	TSW	2011	Rosthern	VI-2	3.4	AA200	3.3	13.3%	-6.92	(CDC Striker)
Q7-1	TSW	2010	Rosthern	VII-1	4.1	AA135	3.3	7.5%	7.00	(Carrera)
Q7-2	AULPC	2010	Rosthern	VII-3	3.9	AD135	2.9	13.4%	7.70	(Carrera)
Q7-2	AULPC	2011	Saskatoon	VII-3	5.8	AD135	3.3	18.3%	12.00	(Carrera)
Q7-2	SS	2010	Saskatoon	VII-3	3	B14	2.4	9.4%	0.12	(Carrera)
Q7-2	SS	2010	Rosthern	VII-3	4.7	AD135	3.4	14.5%	0.18	(Carrera)
Q7-2	SS	2011	Saskatoon	VII-3	5.1	AD135	3.4	11.9%	0.13	(Carrera)
Q7-2	SS	2011	Rosthern	VII-3	4.3	AD165	3.4	13.4%	0.16	(Carrera)

[a] SS= seed shape, MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, AUDPC= area under the disease progress curve calculated based on the four ratings of mycosphaerella blight, LG5= the fifth rating of lodging rated on the date of physiological maturity for individual RILs, Zn= Zinc concentration, Fe= Iron concentration, HT= plant height, Yld= yield, DF= days to flowering, TSW= thousand seed weight, SD= seed dimpling and AULPC= area under the lodging progress curve calculated based on the five ratings of lodging.

[b] Closest marker to the identified QTL with maximum LOD value

[c] Threshold level to declare a QTL significant was determined by performing 1000 permutation test

[d] Percentage of total variability explained by the QTL detected for the trait

[e] The value associated with Carrera allele. A negative value means that the Carrera decreases the value of the trait.

[f] Direction of response is the parent whose additive value of a marker allele increased the value of trait

5.0 Discussion

5.1 Effect of temperature and humidity on the development of mycosphaerella blight

The major factors affecting disease and fruiting body development of *M. pinodes* are temperature and leaf wetness (Huber and Gillespie, 1992). Wet weather and moderate temperatures accelerate disease development (Roger et al. 1999a). An association between temperature and disease development under optimal conditions was described by Roger et al. (1999a). *M. pinodes* spore germination requires a minimum 2h wet period under 15 – 30 ℃ (Roger et al. 1999a). Moisture duration can increase the rate of germination, appressorial formation and germ-tube penetration and thus, the infection process can be shortened (24 - 48h) at temperatures from 15 to 25 ℃ (Roger et al. 1999a). Zhang et al (2005) indicated that humidity is a major factor influencing pycnidia formation in western Canada.

During the growing season (May-August), AUDPC for mycosphaerella blight calculated based on the four ratings was highest at Saskatoon 2010, followed by Rosthern 2010 and 2011, then Saskatoon 2011 (Appendix 5). The possible reason for these phenomena is that Saskatoon received more precipitation in 2010 but less in 2011 than Rosthern (Table 4.1). Banniza et al. (2005) reported that precipitation can increase the severity of mycosphaerella blight ratings at physiological maturity.

The optimum temperature for *M. pinodes* infection is 15-18 ℃ (Wallen, 1965) or 20 ℃ (Bretag, 1991). Mean growing season temperature was greater at Saskatoon in 2010 than in the other three station-years. The temperature ranged from 14.8-16.9 ℃ over the four station-years which is in the optimum range (Wallen and Jeun, 1968). Roger et al. (1999b) reported inoculum pressure becomes higher by mid- to late July, since multiple generations of *M. pinodes* can develop under normal growing conditions due to its relatively short latent period. Lesion expansion which occurs during this period may

result from higher humidity at stages when temperatures are nearly optimum for growth of *M. pinodes* (Roger et al. 1999b).

5.2 Frequency distribution

Transgressive segregation was observed in the RILs frequency distributions for all traits (See 4.2.5). Timmerman-Vaughn et al. (2002) indicated transgressive segregation may result from alleles contributed by both parental lines or from genotype X genotype interactions. The effects could be in either a negative or positive direction (Rieseberg et al. 1999). Genetic gain could be maximized in breeding programs by selecting genetically diverse parents for target loci to create segregating populations (Ubayasena et al. 2010).

5.3 Correlations among phenotypic traits

5.3.1 Relationship between mycosphaerella blight and lodging

Correlation analysis showed that higher levels of disease were correlated with increased lodging scores of pea RILs. Mycosphaerella blight might weaken the stems of infected plants leading to more severe lodging (Conner et al. 2006). Tar'an et al. (2003) also reported that lodging and mycosphaerella blight were positively correlated ($r = 0.35$; $P < 0.01$). Xue et al. (1996) reported that cultivars which were less prone to lodging generally had lower mycosphaerella blight ratings. Mycosphaerella blight severity is increased by lodging by prolonging the period for which the environmental conditions are conducive to infection (Xue et al. 1996). Le May et al. (2001) indicated higher disease severity could be due to denser canopies of certain pea cultivars in terms of differences in microclimate. One way to significantly reduce disease severity was planting peas on a trellis to prevent plants from lodging (Wang, 1998). Conner et al. (2006) reported that lodging accounted for a small portion of the variability associated with differences in mycosphaerella blight severity among cultivars. Banniza et al. (2005)

demonstrated that normal leaf type pea cultivars were prone to lodging and had significantly higher disease levels than semi-leafless cultivars. However, it was not clear whether lodging resistance leading to lower disease development was only due to change in canopy microclimate (Banniza et al. 2005).

5.3.2 Relationship between lodging and plant height

The analysis over 2010 and 2011 indicated lodging (LG5 and AULPC) was positively correlated with plant height ($r= 0.16$, $P<0.001$) and ($r= 0.40$, $P<0.001$), respectively, (Table 4.15) which corresponds to the general understanding. As well, lodging expression is affected by the growing conditions at the pod-filling stage (Obraztsov and Amelin, 1990). Positive correlations between of plant height and lodging were identified in spring wheat (Zuber et al. 1964), tomato (Adelana, 1980), lentil (Erskine and Goodrich, 1988), and soybean (Panthee et al. 2007). Navabi et al. (2006) also found a positive correlation between lodging and plant height in both tall and short wheat genotypic groups. Kelbert et al. (2004) observed that in the short wheat, there was less severity of lodging and less variation for yield and lodging. They indicated that plant height was positively related with lodging at all growth stages and short plants were more tolerant to lodging.

Negative correlation between lodging and plant height was also identified in some other research. For example, Tar'an et al. (2003) found a negative correlation between lodging and plant height ($r = -0.59$; $P<0.001$) based on the pea RIL population derived from MP1401 × Carneval. Taller lines with better lodging resistance than shorter lines were also identified by Knyaz'kova (1987) in a pea F_{10} population.

5.3.3 Relationship between mycosphaerella blight and days to flowering

A negative relationship was found between mycosphaerella blight and days to flowering based on the analysis over 2010 and 2011. Lines with early flowering tended to have greater disease ratings. Zimmer and Sabourin (1986) reported that disease

progress was more severe in older leaves than younger ones due to a decrease in pisatin production in old leaves. Lawyer (1984) also noted that disease severity increased in aged plants. Bretag and Ramsey (2001) reported that plant earliness can accelerate the susceptibility to mycosphaerella blight.

5.3.4 Relationship between mycosphaerella blight, AUDPC and days to maturity

Negative correlations were identified between mycosphaerella blight, AUDPC and days to maturity. Lines with earlier maturity tended to have greater disease ratings. As pea plants mature, severity of mycosphaerella blight increased during field epidemics (Kraft et al. 1998). Conner et al. (2006) found that low AUDPC values were detected in certain late-maturing lines and most late-maturing cultivars had a form of tissue-specific resistance. Bretag et al. (1995) reported that early-maturing cultivars generally had more severe mycosphaerella blight and higher yield losses than late-maturing cultivars. Porta-Puglia et al. (1994) indicated a strong correlation between crop maturity and infection by *M. pinodes*, but no supporting results were provided. Garry et al. (1996) reported a rapid senescence of infected tissue after being infected by *M. pinodes*, speeding up plant maturity. Timmerman-Vaughan et al. (2004) suggested that lower disease scores were recorded in late maturing progeny lines compared with early maturing lines scored on the same date.

5.4 Micronutrients (Se, Zn, and Fe)

Selenium concentration of parents and RILs was much lower at Rosthern compared to Saskatoon, where Se concentration was about five to ten times higher (Fig.4.3). This result was consistent with results reported by Thavarajah et al. (2008, 2010) indicating growing location in western Canada was the major contributor to Se concentration in lentil and pea. In 2005 and 2006, mean Se concentration of 19 lentil genotypes grown at Saskatoon were 4.5 and 10 times greater than at Rosthern,

respectively (Thavarajah et al. 2008). Mean Se concentration of 17 pea cultivars grown at Saskatoon were 2.2 and 7 times greater than at Rosthern in 2005 and 2006, respectively (Thavarajah et al. 2010). Se reaches the food chain through plants which absorb it from the soil. Low soil content of Se has been identified as one of the major influences for Se deficiency (Reilly, 1996). The effect of location in Saskatchewan for Se absorption was greater than the effect of cultivar in lentil and pea (Thavarajah et al. 2010). Gawalko et al. (2009) also reported that the effect of location was more influential on Se concentration than pea cultivar in western Canada. The availability of Se is significantly affected by aeration, water availability, pH and soil texture as well as composition (Combs, 2001; Thavarajah et al. 2007). Se is insoluble and unavailable to plants in soils with poor aeration. Acid soils and complexation with iron or aluminum can also lead to reduction of Se uptake by plants (Reilly, 1996). Se occurs mainly as insoluble selenides and elemental forms (Thavarajah et al. 2008). Based on soil testing (ALS Laboratory Group Agricultural Services, Saskatoon, SK) in spring 2010, soil pH was 6.9 at Saskatoon (clay loam) and 6.7 at Rosthern (silt loam), i.e., the pH and soil texture at the two locations were similar. In the current study, precipitation did not have a significant effect on Se absorption. Se concentration was even higher at Saskatoon in 2011 than in 2010, even though precipitation at Saskatoon 2010 was about twice than that in 2011 (Table 4.1 and Appendix 5). Hence, differences in soil Se concentration and pea genotype could be the main reasons for the variation in Se uptake.

Zinc is absorbed as a form of Zn^{2+} by plant roots (Havlin et al. 2005). Soil factors can affect the availability of Zn to plants and control the amount of Zn in the soil solution. These factors include the total Zn concentration, organic matter concentration, clay concentration, calcium carbonate concentration, microbial activity in the rhizosphere, soil moisture status, concentrations of other trace elements, and concentrations of

macro-nutrients, especially phosphorus and climate (Alloway, 2008). Soil pH is another main factor influencing Zn distribution in the soil, since above-neutral pH made this element readily absorbed (Broadlely et al. 2007; Havlin et al. 2005), however, soil pH was similar at the two locations. By comparing 2010 and 2011 (Appendix 5), Zn concentration may have varied based on differences in precipitation. Zn uptake was increased due to the increased transpiration rate through mass flow in wheat (Grifferty and Barrington, 2000). Hence, soil Zn concentration, soil moisture, soil texture and pea genotype could be the main reasons causing the variations in Zn concentration.

Iron concentrations varied between the two locations following the same trends as Zn (Appendix 5). Fe deficiency happens in soils with high pH, in calcareous soil and in soilless medium. Calcareous soil contains high concentration of calcium carbonate and typically has pH of 8 or greater. Since the soil pH at Saskatoon was 6.9 and at Rosthern was 6.7, differences in Fe availability between locations is expected to be minimal. Excess of phosphorus and calcium in the soil can reduce Fe translocation in the plant (Sainju et al. 2003). Hence, soil Fe concentration, soil moisture, soil texture and pea genotype could be the main reasons causing the variations in Fe concentration.

5.5 Linkage map

The total coverage of the linkage map generated in this study is 288.3 cM which is smaller than the maps previously published for pea, ranging from 899.9 cM to 2416 cM (McCallum et al. 1997; Laucou et al. 1998; Irzykowska et al. 2001; von Stackelberg et al. 2003; Tar'an et al. 2003; Timmerman-Vaughan et al. 2002, 2004; Loridon et al. 2005; Aubert et al. 2006; Fondevilla et al. 2008; Ubayasena et al. 2010). The average distance between two markers in the genetic linkage map in this study is 5.2 cM. The pea SSR markers used in this study were the same as those used in Ubayasena et al. (2010) who reported a map of 899.9 cM based on a RIL population derived from Orb X CDC Striker.

Differences in linkage map coverage result from differences in linkage intensity in different crosses (Laucou et al. 1998). In addition, close genetic relationship between two parental lines could be another reason for low frequency of SSR marker polymorphism and low linkage map coverage (Ubayasena et al. 2010). Differences in linkage map coverage could arise from different mapping populations, mapping strategies, the number of mapping loci and the choice of mapping software (Li et al. 2008). Lack of sufficient markers could be the reason for linkage groups splitting into several fragments (Kosterin and Rozov, 1993). In the current study, the small linkage map coverage could possibly be due to close genetic relationship between the parents (Carrera and CDC Striker) which caused low frequency of SSR marker polymorphism and lead to insufficient markers.

5.6 QTLs for phenotypic traits

5.6.1 QTLs for mycosphaerella blight resistance

The Q3-1 associated with mycosphaerella blight resistance was located in the region between marker AA491 and AA278 on LGIII. QTLs associated with resistance to mycosphaerella blight in pea on LGIII in previously published articles are listed in Appendix 6. By aligning the current linkage map with the map reported by Loridon et al. (2005), the region between marker AA491 and AA278 associated with mycosphaerella blight resistance was located in the middle of LG III. The Q3-1 found in the current study may coincide with the QTL *mpIII-5* for resistance to mycosphaerella blight in the middle of LGIII (Prioul et al. 2004). This QTL *mpIII*-5 with the closest marker AA374a which was only specific for resistance to mycosphaerella blight under field conditions was located near marker AA278 in the middle of LG III (Prioul et al. 2004). QTLs associated with mycosphaerella blight were also identified in Timmerman-Vaughan et al. (2002) distributing on seven LGs on I, II, III, IV, V, VII and A based on an F_2 pea

population derived from a cross between moderately resistant parent 3148-A88 and a susceptible parent Rovar. The location of QTLs on LG III was unknown and was designated *Asc3.1.* Tar'an et al. (2003) identified QTLs on LGII (*ccta2*), LG IV (*cccc1*) and LG VI (*acct1*) associated with reaction to mycosphaerella blight based on the pea RIL population developed from a cross between Carneval (mycosphaerella blight resistant parent) and susceptible parent MP1401. Prioul et al. (2004) identified QTLs for resistance to mycosphaerella blight on LGs III, Va, VI and VII at either seedling or adult stages based on a pea RIL population derived from a cross between DP (partially resistant) and JI296 (susceptible). As well, two QTLs, *mpIII-1* and *mpIII-3*, on LG III were associated with mycosphaerella blight resistance at both seedling and adult stages. These two QTLs were located on the distal part of LG III (Prioul et al. 2004) which was also reported to carry other pea disease resistance genes such as *Rmp4* gene involved in stem resistance to *M. pinodes* in pea seedlings (Clulow et al. 1991b) and *fw* gene, resistant to *Fusarium oxysporum* f. sp. *pisi* race (Weeden et al. 1998). Fondevilla et al. (2008) identified QTLs on LGs II, III, IV, and V under growth chamber or field conditions based on a RIL population derived from a cross between the wild *Pisum sativum* subsp. *syriacum* accession P665 (resistant) and Messire (susceptible). Two QTLs *MpIII.1* and *MpIII.2* were common for growth chamber and field resistance, while *MpIII.3* was specific for adult plant resistance in the field. Fondevilla et al. (2008) indicated that *MpIII.2* and *MpIII.3* were both located in LG III and close to M27 and P202, respectively and these two QTLs may coincide with the QTLs for resistance to ascochyta blight *Asc 3.1* (Timmerman-Vaughan et al. 2002) and *mpIII-3* (Prioul et al. 2004). Marker P202 was also located on the distal part of LG III (Prioul et al. 2004; Fondevilla et al. 2008), indicating the distal part of LG III could contain several genes and play a vital role for resistance to pea diseases (Prioul et al. 2004).

5.6.2 QTLs for lodging resistance and plant height

The current study identified a major QTL Q3-1 associated with lodging resistance and plant height on LG III. By aligning the current linkage map with the map reported by Loridon et al. (2005), the region between marker AA491 and AA278 associated with lodging resistance and plant height was located on the middle of LG III. LG III was also found to be associated with several morphological and physiological characters such as dwarfing gene *le*, controlling short internodes (Sherriff et al. 1994) and the flowering gene *Dne* (Rameau et al. 1998) which causes basal lateral branching under short days (Murfet and Reid, 1993). Tar'an et al. (2003) also identified major loci associated with lodging resistance (*cacc4*) and plant height (*cttg7*) on the LGIII based on their RIL population derived from Carneval (lodging resistant and taller parent) and MP1401. In addition, Tar'an et al. (2003) suggested that QTL associated with lodging resistance and plant height may be linked to a different gene(s) that could improve lodging resistance, because loci for lodging and plant height in their map were located separately from the *Dne* locus (Rameau et al. 1998; Tar'an et al. 2003). However, it has not yet been confirmed whether QTLs on LGIII associated with lodging resistance and plant height in the current study share the same region with the QTLs on LGIII identified by Tar'an et al. (2003). QTLs (*ht1*, *ht2* and *ht3*) associated with plant height were also identified by Prioul et al. (2004) located on LGs II, III and VII. In the current study, the region containing QTLs associated with lodging resistance and plant height was approximately the same as the QTLs for mycosphaerella blight resistance. This result was similar to the result reported by Prioul et al. (2004) that QTLs (*ht1* and *ht2*) associated with plant height were mapped to the same region as the resistance to QTLs *mpII-1* and *mpIII-1*, respectively.

5.6.3 QTLs for micronutrients (Zn and Fe)

By comparing the two parents, Carrera had significantly greater Zn and Fe concentration than CDC Striker based on the average of two locations and two years, suggesting Carrera contains loci associated with increased accumulation of both micronutrients. Q3-1 associated with Zn concentration was derived from Carrera, while Q3-1 associated with Fe concentration was derived from CDC Striker. Thus, it can be concluded genes derived from CDC Striker can enhance the accumulation of Fe. QTLs associated with Zn concentration were co-localized with the regions associated with Fe concentration on LG III. Thus, it is possible that genotypes with higher Zn concentration can have higher Fe concentration. The lines with the greatest Zn and Fe concentrations had about 1.5 times higher concentration than the lowest lines. It is also encouraging from a pea breeding point of view that high Zn and Fe concentration were observed in different environments and the genotype X environment interactions were not significant. Similarly, wheat grains which had higher Fe concentration were also higher in Zn concentration (Graham et al. 1999). Graham et al. (1999) also identified the common mechanisms regulating Zn and Fe accumulation. QTLs for micronutrient concentration in pea have not been reported previously in scientific articles. Molecular level (QTL) information on micronutrients was also reported by Garcia-Oliveira et al. (2009) who identified 31 QTLs associated with mineral elements including Zn and Fe uptake in rice introgression lines. Stangoulis et al. (2007) identified QTLs for Zn and Fe concentration in rice based on a doubled-haploid population, and Deniau et al. (2006) and Assunção et al. (2006) identified QTLs for Zn in a hyperaccumulator plant *Thlaspi caerulescens*.

5.6.4 QTLs for seed quality traits and grain yield

The market value of the harvested crop is affected by the visual quality of pea seeds. Seed shape and seed coat texture are important visual quality traits (Salas et al. 2006). McPhee (2007) stated that one of the most important objectives in pea breeding is improvement of seed quality.

In the current study, QTLs associated with seed shape were located on LGs I (Q1-1) and VII (Q7-2). Ubayasena et al. (2010), in the first report studying QTLs associated with seed shape in field pea, identified six QTLs from the Alfetta X CDC Bronco RIL population on LGs I and VII, and nine QTLs on LGs I, IV and A from the Orb X CDC Striker RIL population. QTLs in this study were mainly concentrated on LG VII. Even though CDC Striker is the common donor parent for roundness in the current study and the study reported by Ubayasena et al. (2010), no common markers were identified associated with seed shape, probably due to the polymorphic status at these loci between Orb, Carrera and CDC Striker in the two RIL populations.

Q5-1 associated with seed dimpling across all environments in this study was located on LG V. In the study of Ubayasena et al. (2010), only one QTL was detected in the Alfetta X CDC Bronco RIL population on LG VII and eight QTLs were detected in Orb X CDC Striker on LGs I, IV, A and D. No common LGs were identified by comparing these three populations, even though the donor parent (CDC Striker) for smooth seed coat in the current study was the same as the RIL population from Orb X CDC Striker. This could be due to marker scarcity (Ubayasena et al. 2010) on LGs I, IV, A and D, or parents Carrera and CDC Striker carrying the same allele at these loci. The QTL associated with seed dimpling on LG V could be given high priority for breeding since it was identified across all environments.

QTLs associated with thousand seed weight were located on LGs III (Q3-1), IV (Q4-1), VI (Q6-1) and VII (Q7-1) in each environment. QTLs associated with seed

weight were also identified by Timmerman-Vaughan et al. (1996). Three QTLs were located on LGs III/IV (due to one common marker), IV and a small group from Primo X OSU442-15 and four regions on LGs I, III and VII, as well as a significant association with three unlinked markers from JI1794 X Slow. LGs III and VII are the common LGs carrying QTL for seed weight between the current study and Timmerman-Vaughan et al. (1996), but no common markers were detected. One region associated with seed weight in JI1794 X Slow corresponded with a lentil genomic region associated with seed weight. Shared regions of linked-group homology in pea and lentil have been identified by Weeden et al. (1992) and Simon et al. (1993) based on comparative mapping studies. *Pgm-c* which was significantly associated with seed weight in a lentil cross (Tahir et al. 1994) was also significantly associated with seed weight in pea (Weeden et al. 1993). Regions of LG VII mapping QTLs associated with seed weight in pea were similar to those regions in lentil (Timmerman-Vaughan et al. 1996).

Two QTLs associated with grain yield were located on LG III based on data from Saskatoon in 2010 and Rosthern in 2011. QTLs were only detected in two out of four environments, indicating environmental effects influenced yield, as similarly reported by other authors, for example, Santalla et al. (2001). QTLs associated with grain yield in pea were also identified by Tar'an et al. (2004). QTLs were identified on LG II (*gccc3*), VI (*gcta2*) and VII (*cctc3* and *cagg9*) based on a RIL population from Carneval X MP1401. No common LGs were identified between the current RIL population Carrera X CDC Striker and Carneval X MP1401. This could be due to marker scarcity on LGs II, VI and VII, or the parents Carrera and CDC Striker carry the same alleles at these loci.

5.6.5. QTLs for days to flowering

Carrera flowered earlier than CDC Striker (Appendix 4; Fig. 4.5). A QTL (Q3-1) associated with days to flowering was located on LG III. One QTL detected at Saskatoon

in 2011 was derived from Carrera. The QTL detected at Rosthern in 2011 was derived from CDC Striker, indicating the existence of some alleles promoting earliness in CDC Striker. Fondevilla et al. (2008) described two QTLs (*dfIII.1 and dfIII.2*) on LG III, one on LG II (*dfII.1*) and one on LG VI (*dfVI.1*) based on a RIL population derived from a cross between the wild *Pisum sativum* subsp. *syriacum* accession P665 (late flowering) and Messire (early flowering). Two QTLs (*dfIII.1and dfII.1*) derived from late flowering parent P665. Prioul et al. (2004) also identified three QTLs (*flo1*, *flo2*, *flo3*) controlling flowering date located on LG II, III, and VI, respectively. Fondevilla et al. (2008) indicated QTL *dfIII.2* located on LG III was in the similar position as the gene *Hr'*, responding highly to photoperiod (Blixt, 1974) and *flo2*, controlling flowering date (Prioul et al. 2004).

In the current study, Q3-1 affecting days to flower was mapped to the same region as resistance to mycosphaerella blight. Similar results were also found by Fondevilla et al. (2008) and Prioul et al. (2004). Fondevilla et al. (2008) found the main QTL (*dfIII.2*) controlling flowering date was co-localized with the QTL (*MpIII.3*) for resistance to mycosphaerella blight. Prioul et al. (2004) identified QTLs (*flo2* and *flo3*) associated with days to flowering were mapped to the same regions as QTLs for mycosphaerella blight resistance (*mpIII-3* and *mpVI-1*) located on LG III and LG VI, respectively. In this case, alleles for resistance to mycosphaerella blight might be associated with alleles that delayed the flowering date. This theory could be explored based on the relationships between mycosphaerella blight, AUDPC and days to flowering (Discusion 5.3.3 and Table 4.13 & 4.14 & 4.15).

5.7 QTLs on LGIII

The region with the most markers linked to QTLs associated with key traits was located on LGIII. Closest marker AA278 deviated from 1:1 Mendelian segregation ratio

(segregation distortion) and might be one of the reasons for these QTLs locating in the same region. These systematic deviations from the expected segregation ratio could be caused by many factors such as errors in marker genotyping and statistical analysis, residual heterozygosity in parental lines (Sibov et al. 2003; Cloutier et al. 1995), or a mutation within the binding site for a DNA marker (Smith et al. 1997). However, Lorieux et al. (1995a) reported segregation distortion had less effect on the estimation of recombination fraction in co-dominant markers. By removing marker AA278 from the linkage map, the order of the rest of the markers was not changed and the region between AA491 and AB44 previously located by these QTLs was still the same. As well, QTLs associated with mycosphaerella blight resistance and lodging resistance located on LG III were also reported in other research (Discussion 5.6.1 & 5.6.2), which support the findings in the current study. The region between AA491 and AB44 appears to harbour multiple genes associated with important traits and further markers should be added in this region.

Lack of sufficient markers is one of the major reasons for the short length of linkage groups in this study. This could be a reason for not discovering QTLs in other genomic regions. The power of QTL detection is reduced for distant markers, hence increasing marker density is important to increase the power of estimation for QTL location (Xu, et al. 2005). Bernardo (2008) stated that the estimation of marker effects will become more refined and marker based selection will become more effective over time with the accumulation of large amounts of marker and phenotypic data.

6.0 CONCLUSIONS and FUTURE RESEARCH

The hypotheses tested in this research were that genomic regions associated with mycosphaerella blight resistance, lodging resistance and micronutrient concentration will be found in the pea cultivars Carrera and CDC Striker and these will be located by

QTL mapping the Carrera/CDC Striker recombinant inbred line population. These hypotheses were accepted.

The objectives of this study were to determine the genetic control of mycosphaerella blight, lodging, and micronutrient accumulation in field pea by genotyping and phenotyping a recombinant inbred line population segregating for these traits and to identify associated quantitative trait loci. These objectives were met. QTLs associated with mycosphaerella blight and lodging resistance as well as zinc and iron concentration were identified. These QTLs will assist breeders to develop useful markers for these traits.

In a collaboration between CDC, University of Saskatchewan and Plant Biotechnology Institute (NRC), SNP markers will be screened between the parents and RILs of the Carrera X CDC Striker population in the near future. SNP markers will be added to this linkage map to increase marker density and refine the identified QTL regions. New QTL regions might also be discovered by increasing marker density. Successful identification of markers linked to QTLs in dense linkage maps can facilitate the pyramiding of target loci. After expanding the length of this map by adding SNP markers, marker assisted selection could be applied if markers are linked tightly to QTLs. This would accelerate the process of selection in early generations of pea breeding.

Recommended future research related to this study includes the following.

1) Phenotyping of this RIL population at new locations will increase the reliability of the identified QTLs.

2) Phenotyping of this RIL population at the same locations (Saskatoon and Rosthern) will strengthen the phenotypic estimates of each trait allowing more precise QTL analysis.

3) Efficient markers (based on SSRs or SNPs) associated with key traits will be

developed after increasing the marker density of the map.

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APPENDICES

Appendix 1. Average, minimum, maximum values and coefficient variation (CV) for mycosphaerella blight and AUDPC collected in 2010, 2011 and across both years for the parental pea cultivars Carrera and CDC Striker and the recombinant inbred lines derived from the cross between these two cultivars. The values were based on least square means.

2010	MB1	MB2	MB3	MB4	AUDPC
Carrera	2.3	5.3	7.5	8.8	198.6
CDC Striker	1.5	4.0	5.8	7.5	151.4
Population Mean	2.3	4.7	7.1	8.4	186.8
Minimum	1.5	4.0	5.2	7.0	160.1
Maximum	3	5.5	8.4	9.0	210.0
CV (%)	17	8	8	6	6

2011	MB1	MB2	MB3	MB4	AUDPC
Carrera	2.0	4.3	7.5	7.8	179.4
CDC Striker	1.0	3.5	6.5	6.5	148.8
Population Mean	1.5	4.0	7.0	7.5	167.2
Minimum	1.0	3.2	5.8	6.5	147.0
Maximum	2.5	4.7	8.0	8.5	184.6
CV (%)	24	8	6	6	5

2010&2011	MB1	MB2	MB3	MB4	AUDPC
Carrera	2.1	4.8	7.6	8.3	189.0
CDC Striker	1.3	3.8	6.1	7.0	150.1
Population Mean	1.9	4.4	7.1	8.0	177.0
Minimum	1.3	3.6	5.9	6.9	154.0
Maximum	2.8	5.0	8.2	8.6	193.4
CV (%)	18	6	6	5	4

Notes: MB1= the first rating of mycosphaerella rated one week after flowering, MB2= the second rating of mycosphaerella blight rated two weeks after the first rating, MB3= the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity, MB4= the fourth rating of mycosphaerella blight rated on the date of physiological maturity for individual RILs, AUDPC= area under disease progress curve based on all the four ratings of mycosphaerella blight, four ratings were based on 0= no disease to 9= severely blighted.

Appendix 2. Average, minimum, maximum values and coefficient variations (CVs) for lodging and AULPC collected in 2010, 2011 and combined from both years for the parental pea cultivars Carrera and CDC Striker and the recombinant inbred lines derived from the cross between these two cultivars. The values were based on least square means.

2010	LG1	LG2	LG3	LG4	LG5	AULPC
Carrera	1.0	1.3	2.3	7.3	8.3	115.5
CDC Striker	1.0	1.0	2.0	5.8	6.0	92.8
Population Mean	1.2	1.5	3.6	6.0	6.8	126.1
Minimum	1.0	1.0	1.3	3.8	4.8	89.8
Maximum	3.3	4.3	5.0	8.3	8.8	222.8
CV (%)	39	51	30	15	12	14

2011	LG1	LG2	LG3	LG4	LG5	AULPC
Carrera	2.3	2.8	3.0	7.3	7.8	135.6
CDC Striker	2.0	2.0	3.0	4.8	5.3	105.9
Population Mean	2.2	2.6	3.2	5.1	6.3	186.2
Minimum	1.0	1.0	1.5	3.5	4.0	89.4
Maximum	4.5	5.0	5.3	7.2	8.0	341.6
CV (%)	35	33	24	15	14	19

2010&2011	LG1	LG2	LG3	LG4	LG5	AULPC
Carrera	1.6	1.9	2.6	7.3	8.0	125.6
CDC Striker	1.5	1.6	2.5	5.3	5.6	99.3
Population Mean	1.7	2.1	2.9	5.8	6.5	156.2
Minimum	1.0	1.0	1.6	4.0	5.0	91.3
Maximum	3.4	4.4	4.9	7.4	8.1	262.6
CV (%)	33	37	24	11	10	15

Notes: LG1= the first rating of lodging rated one week after flowering, LG2= the second rating of lodging rated two weeks after the first rating, LG3 = the third rating of lodging rated one week after the second rating, LG4= the fourth rating of lodging rated when the first RILs reached physiological maturity, LG5= the fifth rating of lodging rated at physiological maturity for individual RILs, AULPC= area under the lodging progress curve calculated based on the five ratings of lodging, five lodging ratings were based on 1= upright to 9= completely flat.

Appendix 3. Average, minimum, maximum values and coefficient variations (CVs) for Zn and Fe collected in 2010, 2011 and combined from both years for the parental pea cultivars Carrera and CDC Striker and the recombinant inbred lines derived from the cross between these two cultivars. The values were based on least square means.

	Zn(2010)	Zn(2011)	Zn (Combined)	Fe (2010)	Fe(2011)	Fe (Combined)
Carrera	34.3	29.1	31.7	48.8	48.0	48.3
CDC Striker	27.6	26.3	27.0	42.0	41.0	41.5
Population Mean	33.2	28.7	30.9	48.5	45.4	47.0
Minimum	27.7	23.7	25.7	42.1	39.5	41.2
Maximum	39.6	34.8	37.3	59.0	56.4	57.4
CV (%)	10	8	8	7	6	7

Notes: Zn= zinc concentration (ppm) and Fe= iron concentration (ppm).

Appendix 4. Average, minimum, maximum values and coefficient variations (CVs) for other phenotypic data collected in 2010, 2011 and combined across two years (not for SD) for the parental pea cultivars Carrera and CDC Striker and the recombinant inbred lines derived from the cross between these two cultivars. The values were based on least square means.

2010	DF	DM	PS	HT	Yld	TSW	SS	SD
Carrera	50	97	6.9	55	542	265	3.3	10
CDC Striker	55	95	6.0	68	524	239	2.6	1
Population Mean	54	98	7.0	64	502	260	3.2	8
Minimum	48	92	4.5	46	185	218	2.2	1
Maximum	57	100	8.5	83	702	311	4.0	40
CV (%)	6	3	17	17	25	11	15	93

2011	DF	DM	PS	HT	Yld	TSW	SS	SD
Carrera	55	99	6.9	55	433	281	3.1	14
CDC Striker	57	98	7.7	72	473	230	2.6	1
Population Mean	57	99	6.7	66	398	256	2.9	8
Minimum	55	95	3.8	45	151	216	2.0	1
Maximum	59	103	8.5	96	589	312	3.7	40
CV (%)	2	3	17	17	25	10	15	95

2010&2011	DF	DM	PS	HT	Yld	TSW	SS
Carrera	53	98	6.8	55	487	273	3.2
CDC Striker	56	96	6.8	70	498	234	2.6
Population Mean	55	98	6.8	64	451	259	3.0
Minimum	52	94	4.8	49	176	217	2.2
Maximum	58	101	8.3	83	614	312	3.8
CV (%)	5	3	17	20	22	10	16

Notes: DF= days to flowering (days), DM= days to maturity (days), PS= plant stand based on 1= poor to 10= excellent stand, HT= plant height (cm), Yld= yield (g m^{-2}), TSW= thousand seed weight (g), SS= seed shape based on 1= round to 5= blocky, and SD= percentage of seeds with dimpling.

Appendix 5. Least square means of 142 recombinant inbred line population derived from Carrera/CDC Striker for area under the disease progress curve based on the four ratings of mycosphaerella blight (AUDPC), selenium (Se) concentration, zinc (Zn) concentration and iron (Fe) concentration at Saskatoon and Rosthern in 2010 and 2011.

Location/ Mean	AUDPC	Se (ppm)	Zn (ppm)	Fe (ppm)
2010 Saskatoon	187.2	1.8	35.2	52.8
2010 Rosthern	186.4	0.3	31.1	44.5
2011 Saskatoon	155.8	2.5	26.9	43.5
2011 Rosthern	178.7	0.5	30.4	47.3
Standard deviation	14.6	1.1	3.4	4.1

Appendix 6. QTLs for mycosphaerella blight resistance on LGIII identified in previously published papers and in the current study with trait, closest mark, LOD and R^2.

QTLs	Trait	Closest marker	LOD	R^2 (%)	References
Asc 3.1		PI39	2.9	10	Timmerman-Vaughan et al. 2002
mpIII-1	CC/FC	E08-980	13.0/12.5	18/26	Prioul et al. 2004
mpIII-2	CC	PSP40SG	4.8	7	Prioul et al. 2004
mpIII-3	CS/FC	V03-1000	4.9/3.9	6/7	Prioul et al. 2004
mpIII-4	FS	F09-1900	6.8	29	Prioul et al. 2004
mpIII-5	FS	AA374a	5.8	11	Prioul et al. 2004
MpIII.1	DRseedl	$OPW5_{387}$	6.4	17	Fondevilla et al. 2008
MpIII.1	DS (2005)	OPM6598/$OPW5_{387}$	4.3	29	Fondevilla et al. 2008
MpIII.1	DRst (2006)	$OPW5_{387}$	3.2	9	Fondevilla et al. 2008
MpIII.2	DRseedl	$OPM15_{431}$	3.5	9	Fondevilla et al. 2008
MpIII.2	DRst (2005)	$OPB11_{1477}$	4.3	14	Fondevilla et al. 2008
MpIII.3	DR1 (2005)	$OPAI14_{1353}$/$OPW2_{1157}$	8.5	31	Fondevilla et al. 2008
MpIII.3	DRst (2005)	$OPAI14_{1353}$	10.0	42	Fondevilla et al. 2008
MpIII.3	DS (2005)	$OPAI14_{1273}$/$OPAI14_{1353}$	10.8	46	Fondevilla et al. 2008
MpIII.3	DR1 (2006)	$OPAI14_{1353}$/$OPW2_{1157}$	15.7	52	Fondevilla et al. 2008
MpIII.3	DRst (2006)	$OPAI14_{1353}$	10.2	34	Fondevilla et al. 2008
MpIII.3	DS (2006)	$OPAI14_{1353}$	11.5	32	Fondevilla et al. 2008
Q3-1	MB32010SAS	AA491	5.5	16	Current study
Q3-1	MB32010ROS	AA278	7.6	19.7	Current study
Q3-1	MB32011SAS	AA491	5.6	14.2	Current study
Q3-1	MB32011ROS	AA278	3.5	7.6	Current study
Q3-1	AUDPC2010ROS	AA278	3.4	9.5	Current study
Q3-1	AUDPC2011SAS	AA278	4.9	13.1	Current study

Notes: CC = stipules and stems at the seedling stage under controlled conditions; CS = stems at the seedling stage under controlled conditions; FC = stipules and stems at the adult plant stage in the field; FS = stems at the adult plant stage in the field; DRseedl = disease rating in leaves of seedlings scored under growth chamber conditions; DRl = disease rating on leaves scored under field conditions; DRst = disease rating on stems scored under field conditions; DS = disease severity (percentage of the plant area covered by symptoms) estimated under field conditions; MB32010SAS = the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity at Saskatoon in 2010; MB32010ROS = the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity at Rosthern in 2010; MB32011SAS = the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity at Saskatoon in 2011; MB32011ROS = the third rating of mycosphaerella blight rated when the first RILs reached physiological maturity at Rosthern in 2011; AUDPC2010ROS = area under the disease progress curve based on four ratings of mycosphaerella blight at Rosthern in 2010; AUDPC2011SAS = area under the disease progress curve based on four ratings of mycosphaerella blight at Saskatoon in 2011.